

ANNUAL REPORT

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1981 - September 30, 1982

VOLUME II

INDIVIDUAL PROJECT REPORTS

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| Z01 MH 01565-01 SMRP | Regulation of GABA _A and GABA _B Receptor Function..... | 1139 |
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INTRAMURAL RESEARCH PROGRAM
NATIONAL INSTITUTE OF MENTAL HEALTH

RESEARCH PROJECT SERIAL NUMBER LISTING:

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| Z01MH00021 | Z01MH00276 | Z01MH00489 |
| Z01MH00034 | Z01MH00277 | Z01MH00491 |
| Z01MH00035 | Z01MH00326 | Z01MH00495 |
| Z01MH00036 | Z01MH00327 | Z01MH00500 |
| Z01MH00037 | Z01MH00328 | Z01MH00501 |
| Z01MH00039 | Z01MH00329 | Z01MH00502 |
| Z01MH00040 | Z01MH00330 | Z01MH00503 |
| Z01MH00041 | Z01MH00331 | Z01MH00504 |
| Z01MH00049 | Z01MH00332 | Z01MH00505 |
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| Z01MH00100 | Z01MH00382 | Z01MH00871 |
| Z01MH00111 | Z01MH00388 | Z01MH00881 |
| Z01MH00112 | Z01MH00394 | Z01MH00882 |
| Z01MH00114 | Z01MH00396 | Z01MH00887 |
| Z01MH00117 | Z01MH00397 | Z01MH00889 |
| Z01MH00124 | Z01MH00401 | Z01MH00900 |
| Z01MH00132 | Z01MH00402 | Z01MH00901 |
| Z01MH00147 | Z01MH00403 | Z01MH00902 |
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| Z01MH00271 | Z01MH00484 | Z01MH01035 |
| Z01MH00274 | Z01MH00486 | Z01MH01037 |
| Z01MH00275 | Z01MH00488 | Z01MH01038 |

RESEARCH PROJECT SERIAL NUMBER LISTING (Cont.):

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| Z01MH01039 | Z01MH01562 |
| Z01MH01081 | Z01MH01563 |
| Z01MH01090 | Z01MH01564 |
| Z01MH01091 | Z01MH01565 |
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| Z01MH01093 | Z01MH01831 |
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| Z01MH01335 | Z01MH01834 |
| Z01MH01337 | Z01MH01836 |
| Z01MH01338 | Z01MH01850 |
| Z01MH01500 | Z01MH01851 |
| Z01MH01503 | Z01MH01852 |
| Z01MH01505 | Z01MH02032 |
| Z01MH01506 | Z01MH02033 |
| Z01MH01508 | Z01MH02034 |
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| Z01MH01514 | Z01MH02132 |
| Z01MH01515 | Z01MH02135 |
| Z01MH01516 | Z01MH02138 |
| Z01MH01518 | Z01MH02139 |
| Z01MH01520 | Z01MH02140 |
| Z01MH01521 | Z01MH02142 |
| Z01MH01524 | Z01MH02143 |
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| Z01MH01536 | Z01MH02149 |
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| Z01MH01540 | Z01MH02152 |
| Z01MH01542 | Z01MH02153 |
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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00092-08 BP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Central Amines and Aggression, Suicide, and Alcoholism | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI Other: | Gerald L. Brown, M.D. Frederick K. Goodwin, M.D. O.L. Royal, M.D. Peter F. Goyer, M.D. William J. Klein, M.D. William E. Bunney, Jr., M.D. | Medical Officer Chief Former Chief, Department of Psychiatry Medical Officer Medical Officer BP NIMH CP NIMH NPMC PPMC NPMC |
| COOPERATING UNITS (if any) Clinical Psychobiology Branch, DCBR, NIMH; Department of Psychiatry, National Naval Medical Center; Portsmouth Naval Medical Center | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Office of the Chief | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: .75 | PROFESSIONAL: .45 | OTHER: .30 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The National Institute of Mental Health (NIMH) and the National Naval Medical Center (NNMC) collaboratively studied <u>central amine</u> metabolites in the <u>cerebrospinal fluid</u> (CSF) of psychi- atric patients. Results to date indicate that aggression and anti-social behav- ior is inversely correlated with CSF 5-hydroxyindoleacetic acid (5HIAA) and posi- tively correlated with cyclic 3',5'-adenosine monophosphate (c-AMP). Low CSF 5HIAA is also associated with suicidal history; suicidal history is also associ- ated with a history of aggressive, anti-social behavior. Findings have been largely replicated on two separate populations. Alcoholics have decreased CSF 5HIAA during abstinence. Disulfiram (Antabuse) appears to lower CSF <u>homovanil- lic acid</u> (HVA) and appears to increase serum <u>norepinephrine</u> (NE); low CSF dopa- mine- <u>b-hydroxylase</u> (DBH), low platelet monoamine oxidase (MAO), low plasma amine oxidase (AO), and high red-cell catechol-O-methyl transferase (COMT) are related to adverse reactions to disulfiram. CSF DBH is inversely related to significant deviations in certain personality measures on the MMPI; CSF 5HIAA is inversely related to the Pd scale. A trivariant relationship exists between history of aggression, history of suicidal behavior, and lower CSF 5HIAA. | | |

Project Description:

Objectives: Evidence obtained in recent years indicate that epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5HT), acetylcholine (Ach) and gamma-amino butyric acid (GABA), among others, act as neurotransmitters and/or neuromodulators of the central nervous system (CNS). Although considerable indirect pharmacologic evidence has linked these amine systems with psychiatric illness (particularly affective illness and schizophrenia), the relative lack of direct data in man has limited the applicability of these linkages to improved diagnosis and treatment of the major psychiatric disorders. Direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. There have been virtually no data on central neurochemical function in the various personality disorders--a rather striking deficit in our knowledge, considering the evidence suggesting that some personality disorders, particularly those involving criminality, have patterns of a genetic component. Furthermore, certain patterns of behavior often seen within personality disorders, i.e. depression, alcoholism, and suicide, also appear to have genetic components. Data from animals suggests a relationship between aggressive behavior and biogenic amines. Neurochemical studies in human alcoholism have also been limited. A purpose of this project is to extend the studies of central amine turnover into larger and more diverse populations of psychiatric patients and to assess behavioral-biochemical relationships that might not be diagnostically specific.

Methods Employed: Independent studies have been a joint effort between the National Naval Medical Center and the National Institute of Mental Health, both in Bethesda, Maryland. Both study groups consisted entirely of military, active duty inpatient males of normal intelligence; the first study was comprised of 26 subjects and the second, 12. More patients were not available for the second study. The two groups were of the same age range (17 to 32 years) and of a similar mean age (mean \pm SD = 22.1 ± 3.6 and 22.0 ± 5.2 , respectively). Height was unavailable in the first study, but ranged from 68 to 73 inches in the second study (70.6 ± 1.4). All study subjects were unpaid volunteers from whom informed consent was obtained. Patients were excluded from both studies if medical disorders were present or if there was evidence of past or current primary affective disorder or schizophrenia, or if other than transient organic brain syndrome had ever been observed. An important clinical difference between the two groups, however, was that any presence or history of psychotic symptomatology was a basis for exclusion from the first study group; whereas, a history of Brief, Reactive Psychosis (DSM-III, No. 298.80) as a secondary diagnosis was present in four of the second study group and two others had had episodes of severe withdrawal sufficient to meet the criteria for Schizoid Personality Disorder (DSM III, No. 301.20) as a secondary diagnosis. Clinical diagnoses and clinical history assessments were made independently of biochemical investigations. Further exclusion criteria were the ingestion of any drug, prescribed or illicit, within ten days of a scheduled lumbar puncture (LP) and heavy use of alcohol (a score of greater than 6 on the Michigan Alcoholism Screening Test [MAST]). Alcoholic study groups were somewhat older and did score greater than 6 on the MAST. They did not have significant histories of medical, affective, schizophrenic, or organic disorders. Material available for evaluating each patient included full psychiatric/medical history, physical examination, and job performance assessments. Since a purpose of admission was evaluation of suitability

lity for further military service, emphasis was given to a life history of aggression, particularly in response to authority. The categories of behavior used to determine aggression history, its scoring, its reliability, and its use in a normal, age-matched, sex-matched control group have been described in detail in published studies. In addition, the Buss-Durkee Inventory (BDI) for aggression and the Minnesota Multiphasic Personality Inventory (MMPI) have been used. Individual items of the psychopathic deviate (Pd) scale of the MMPI approximate behaviors reflected in the life history of aggression measure. The use of standardized personality assessment instruments should facilitate attempts at further replication. All evaluative and behavioral data were collected, scored, and analyzed independently of the biochemical data.

Cerebrospinal fluid (CSF) was obtained from study group subjects following the procedures developed and revised at NIH and elsewhere. Assay details are described in the published studies. Other studies in conjunction with pharmacological interventions have further provided knowledge of functional brain chemistry in relationship to behavior, diagnosis, and personality.

Major Findings: Initial results from a group of personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment indicated that aggressive behavior is inversely correlated with 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with 3-methoxy-4-hydroxyphenylglycol (MHPG). A group of personality disorders have shown no statistically significant difference in CSF cyclic 3', 5'-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from groups of depressive, manic, and schizophrenic patients, though aggressive behavior is positively correlated with c-AMP. Those patients who were administratively discharged from the service and those with prior history of suicidal attempts have shown lower CSF 5HIAA and higher MHPG, c-AMP, and c-GMP. A second group of patients with borderline personalities (DSM-III) show an inverse relationship between CSF 5HIAA and psychopathic deviation (Pd) on the Minnesota Multiphasic Personality Inventory (MMPI) as well as a history of aggressive behavior; the MHPG relationship was not replicated. A trivariant relationship between a history of aggression, history of suicidal behavior, and lower CSF 5HIAA is readily apparent.

Alcoholics have also been studied. It has been found that alcoholics do not differ from personality disorders with respect to CSF HVA. However, studies do indicate that the mean CSF 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in relation to the length of abstinence to reach a baseline value not differing from the personality disorders. An additional study shows that, though HVA values do not change in relationship to the time period post-intoxification-withdrawal, these levels are depressed concomitant with the use of disulfiram (Antabuse), a dopamine- β -hydroxylase (DBH) inhibitor. In addition, disulfiram use correlates with a significant increase in serum NE. Mean serum DBH in alcoholics versus normal controls was significantly lower, blood pressure was significantly higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Other studies indicate that lower CSF DBH is correlated with increasing psychopathology as measured by the MMPI and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore, low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-O-methyl transferase (COMT) are

associated with disulfiram-induced psychoses. Other studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism.

Significance for Mental Health Research: CNS functioning is greatly understudied in some major groups of psychiatric patients, viz. personality disorders, alcoholics, and borderlines. Studies of animal models, as well as Gilles de la Tourette syndrome, hyperactive children, and prisoners suggest a relationship between central neurotransmitter systems and aggressive behavior. Human suicidal behavior has an enormous public health and social significance and, previously, had largely been studied from a psychological, sociological point-of-view only. These studies lead to the possibility of identifying those at risk for anti-social and suicidal behaviors and possibly altering these behaviors through neuropharmacological adjuncts to management of the psychiatric and/or behavioral problems. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also drug-free personality disorders may serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. The progress of the project is submitted to the Navy for reapproval each March and has now been terminated with regard to obtaining new subjects. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC. There is still a significant amount of neurochemical, behavioral, and psychological data to be analyzed and reported from the patients who have participated in these studies.

Publications:

Brown, G.L., Ebert, M.H., Goyer, P.F., Jimerson, D.C., Klein, W.J., Bunney, W.E., Jr., and Goodwin, F.K.: Aggression, suicide, and serotonin relationships to CSF amine metabolites. Am. J. Psychiatry 139: 741-746, 1982.

Brown, G.L., Goodwin, F.K., and Bunney, W.E., Jr.: Human aggression and suicide: their relationship to neuropsychiatric diagnoses and serotonin metabolism. In Ho, B.T., Usdin, E., and Bunney, W.E., Jr. (Eds): Serotonin in Biological Psychiatry. New York, Raven Press, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00021-17 BP |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Studies of Sleep</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: OTHER: | J. C. Gillin W. B. Mendelson R. J. Wyatt N. E. Rosenthal R. J. Loewenstein W. E. Bunney, Jr. D. L. Murphy F. K. Goodwin R. M. Post E. S. Gershon | Research Psychiatrist, Unit on Sleep Studies APB, BP NIMH Research Psychiatrist SMR NIMH Chief, Adult Psychiatry Branch APB NIMH Senior Staff Fellow BP NIMH Clinical Associate APB NIMH Chief BP NIMH Chief CN NIMH Director IRP NIMH Acting Chief BP NIMH Chief, Sec. on Psychogenetics BP NIMH |
| - See Attached Continuation - | | |
| COOPERATING UNITS (if any) Adult Psychiatry Branch, NIMH Division of Special Mental Health Research, St. Elizabeths Hospital, Wash. D.C. - See Attached Continuation - | | |
| LAB/BRANCH <p style="text-align: center;">Biological Psychiatry Branch</p> | | |
| SECTION <p style="text-align: center;">Unit on Sleep Studies</p> | | |
| INSTITUTE AND LOCATION NIMH; ADAMHA; NIH; St. Elizabeths Hospital, Wash. DC 20032 <p style="text-align: center;">NIMH, ADAMHA; NIH; Bethesda, Maryland 20205</p> | | |
| TOTAL MANYEARS: <p style="text-align: center;">9.0</p> | PROFESSIONAL: <p style="text-align: center;">3.0</p> | OTHER: <p style="text-align: center;">10.0</p> |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> This unit, which was jointly administered under W. E. Bunney, Jr., Chief, Bio- logical Psychiatry Branch, and Richard J. Wyatt, Chief, Adult Psychiatry Branch, has long conducted studies on basic and clinical aspects of sleep in man and animals. It has made contributions to the basic neuropharmacological control of sleep and nocturnal neuroendocrinology, clinical use and abuse of sleeping pills, affective illness, schizophrenia, insomnia, childhood enuresis and obses- sive-compulsive disorder, Gille de la Tourette's syndrome, dementia, and adult obsessive-compulsive disorder. Our studies suggest that patients with primary affective illness have a genetically inherited supersensitive cholinergic system both when ill and when in remission. </p> <p> Progress has been made in identifying an endogenous sleep factor in sleep- deprived rat brain. Local glucose utilization is down by approximately 30% throughout the brain during NREM sleep. </p> | | |

Names, Laboratory and Institute Affiliations, and Titles of Principal Investigators and All Other Professional Personnel Engaged on the Project (Cont'd):

| | | | | |
|--------|------------------|---|-----------------|--------|
| OTHER: | H. Weingartner | Research Psychologist | LPP | NIMH |
| | D. P. van Kammen | Chief, Sec. on Neuropsychopharmacology | BP | NIMH |
| | M. H. Ebert | Chief, Sec. on Experimental Therapeutics | LCS | NIMH |
| | J. Rapoport | Chief, Section on Childhood Mental Illness | BP | NIMH |
| | T. A. Wehr | Chief, Clin. Research Unit | CP | NIMH |
| | P. Gold | Chief, Unit on Neuroendocrinology | CP | NIMH |
| | M. S. Buchsbaum | Chief, Section on Clinical Psychophysiology | BP | NIMH |
| | L. Sokoloff | Chief, Laboratory on Cerebral Metabolism | LCM | NIMH |
| | C. Kennedy | Section on Developmental Neurochemistry | LCM | NIMH |
| | M. Mishkin | Chief, Section on Cerebral Mechanisms | LN | NIMH |
| | R. Nakamura | Senior Staff Fellow | LPP | NIMH |
| | S. Rosen | Senior Investigator | CEB | NIAMDD |
| | D. A. Sack | Clinical Associate | CP | NIMH |
| | M. Feinberg | Assoc. Professor of Psychiatry | Univ Mich. | |
| | B. Carroll | Acting Chairman | Univ Mich. | |
| | D. Kupfer | Professor of Psychiatry | Univ Pittsburgh | |
| | T. Insel | Clinical Associate | CN | NIMH |
| | R. Cohen | " " | CN | NIMH |
| | T. Uhde | " " | BP | NIMH |
| | S. M. Paul | " " | CPB | NIMH |
| | P. Skolnick | " " | LBC | NIADDK |
| | K. F. Berman | " " | APB | NIMH |
| | J. Rojas-Ramirez | Visiting Scientist | APB | NIMH |

Cooperating Units (Cont'd):

Laboratory of Psychology and Psychopathology, NIMH
 Clinical Neuropharmacology Branch, NIMH
 Clinical Psychobiology Branch, NIMH
 Section on Psychobiology, BPB, NIMH
 Section on Psychogenetics, BPB, NIMH
 Section on Neuropsychopharmacology, BPB, NIMH
 Section on Experimental Therapeutics, LCS, NIMH
 Unit on Childhood Mental Illness, BPB, NIMH
 Clinical Research Unit, CPB, NIMH
 Section on Clinical Psychophysiology, BPB, NIMH
 Department of Medicine, University of Rochester, Rochester, New York
 Department of Psychiatry, University of Pittsburgh
 Department of Psychiatry, University of Michigan

Project Description:Objectives:

Experimental findings over the past decade promise understanding of the basic biochemical processes underlying sleep; and increasingly clear demonstrations of the severity of sleep disturbances in many psychiatric patients indicate the need for better means of alleviation. To whatever extent understanding of sleep can be achieved, more effective treatment of its disturbances will surely follow.

I. Major Findings: Pharmacological Studies of SleepA. Cholinergic1. Time-dependent Effects of Physostigmine on Normal Human Sleep and Arousal

Physostigmine induces REM sleep or arousal in man depending upon the dose and time of administration. Thirty-three normal volunteers received 40 physostigmine (0.5 mg over 2-3 min) and 40 matched saline (sal) infusions. (1) With postinfusion-arousals excluded, infusions given 5 min after sleep onset reduced time from infusion to onset of REM from 78.9 ± 14 min (sal) to 52.4 ± 10.4 (phy) ($p < 0.05$, two-tailed "t" test). (2) Infusions given 35 min after sleep onset reduced time from infusion to onset of REM from 65.7 ± 15.2 (sal) to 11.2 ± 2.1 (phy) ($p < 0.005$). REM was induced more rapidly by physostigmine infusions given 35 min after than 5 min after sleep onset ($p < 0.01$). (3) For the infusions at REM onset, 5 min after and 25 min after REM, the predominant effect was one of arousal. Judging from number and duration of awakenings, the order of intensity of arousal was: REM > 5 min after > 25 min after REM. Although a dose of 0.5 mg awoke the subjects when given 25 minutes after the first REM period, a dose of 0.25 mg induced REM.

These data suggest that cholinergic mechanisms are involved in initiating REM sleep and arousal. During the first NREM period, physostigmine more readily induces REM when given 35 min than when given 5 min after sleep onset, whereas the same dose given at REM onset and during the second NREM period produces different intensities of arousal.

In order to determine whether the duration of REM could be affected, seven normal male volunteers (mean age 22.8) were studied for three nights (one "adjustment" and two experimental nights). Subjects were pre-treated with 0.75 mg IM methacopolamine prior to bedtime; and during sleep they received either placebo or physostigmine 1.0 mg given by a slow IV drip over a period of 60 minutes. The IV infusion began 35 minutes after sleep onset and ended about 95 minutes after sleep onset. Physostigmine significantly decreased both the latency of the first REM period (placebo = 108.4 ± 16.2 min, mean \pm S.E., physo = 61.7 ± 2.2 min, $p < .05$) and the duration of the second NREM period (placebo = 110.4 ± 13.6 , physo = 74.4 ± 6.5 , $p < .05$). The duration of individual REM periods was not altered. Although total REM time and REM percent showed a non-significant increase of physostigmine (RT = 98.2 ± 6.4 min, R% = $25 \pm 1.5\%$) as compared to placebo nights (RT = 80.9 ± 9.4 , R% = $20.1 \pm 1.9\%$) this can be accounted for by the greater number of REM periods on physostigmine (4.3 ± 0.3) than on placebo (3.6 ± 0.2) conditions. Cholinergic mechanisms may modulate the "timing" rather than the "maintenance" of REM.

2. Physostigmine-induced REM is Associated with Normal Dreaming

To examine the psychological process of dreaming in man, seventeen normal volunteers (11M, 6F, mean age 23), after pretreatment with methscopolamine 0.5 mg IM, slept with an IV needle, and at either 10 or 35 minutes after sleep onset received one intravenous infusion per night of either placebo or physostigmine 0.5 mg.

During Experiment I subjects were given an IV infusion 10 min after sleep onset and were awakened 20 min after infusion. Experiment II included awakening either 7 or 20 min following an infusion given 35 min after sleep onset; in addition subjects were instructed to go back to sleep after the 20 min awakening and were then awakened approximately 7 min after they had spontaneously gone into REM sleep. The above study was designed to control for (1) time of infusion, (2) time from infusion to awakening, (3) state of sleep prior to awakening.

At each awakening a taped interview was conducted. Subjects were questioned waking. After the interview subjects filled out a questionnaire. A dream mentation scale was constructed from the questionnaire ranging from +5 (absolute confidence in presence of dream) to -5 (absence of dreaming).

Comparison of Dream Reports from Physostigmine-induced and Spontaneous First REM Period

| | <u>Physostigmine-induced REM Periods</u> | <u>Spontaneous REM Periods</u> | <u>p</u> |
|--|--|------------------------------------|----------|
| 1) No. of REM awakenings | 9 | 10 | NS |
| 2) No. of dream reports* | 8 | 7 | NS |
| 3) Duration of REM prior to waking (mean \pm SEM min) | 6.6 \pm 0.6 | 7.1 \pm 0.4 | NS |
| 4) REM Density (0-8 scale) | 1.7 \pm 0.2 | 1.7 \pm 0.4 | NS |
| 5) Vividness of dreams* | 3.1 \pm 0.4 | 2.7 \pm 0.4 | NS |
| 6) Unusualness (0-5)* | 1.6 \pm 0.5 | 1.7 \pm 0.3 | NS |
| 7) Emotionality (0-5)* | 2 \pm 0.4 | 1.9 \pm 0.5 | NS |

* These scores were obtained from a questionnaire filled out independently by both subjects and experimenter and from a structured interview conducted by the experimenter.

During Experiment I and the 7 minute post-infusion awakenings of Experiment II, neither REM sleep nor dreaming was induced by physostigmine. In the 20 minute post-infusion, physostigmine induced the onset of REM sleep in approximately 13 minutes in all but one subject. Those drug-induced REM episodes were associated with a significantly higher incidence of dream recall than placebo.

This study indicates that the physostigmine-induced physiological characteristics of REM sleep are accompanied by dreaming. The dreaming occurring during physostigmine-induced REM periods appears to be similar to that observed during spontaneously occurring REM sleep.

3. Induction of REM Sleep in Man by Arecoline, a Cholinergic Muscarinic Agonist: Blockade by Scopolamine

Ten normal volunteers (8M, 2F, mean age 25.3) were each studied on 4 randomly ordered experimental nights (after a night of adaptation). After pretreatment with methscopolamine 0.5 IM on three of the experimental nights, they each received one intravenous infusion about 32 to 35 min after sleep onset of either (1) placebo, (2) arecoline 1.0 mg IV, or (3) arecoline 1.5 mg IV. On the fourth night pretreatment was scopolamine 0.5 mg IM followed by IV infusion of arecoline 1.5 mg. The results were: (1) Both arecoline 1.0 mg and 1.5 mg triggered the onset of REM sleep, 20.1 and 15.9 min after infusion, respectively. (2) Pretreatment with scopolamine blocked the REM-inducing effect of arecoline 1.5 mg IV. (3) Once the onset of the first REM period was moved forward, subsequent nonREM-REM cycles were advanced without altering REM-REM intervals. (4) The number of REM periods was significantly increased after arecoline 1.0 mg (4.1±0.3) and 1.5 mg (4.5±0.2) compared to placebo (3.5±0.2). (5) Total REM time and percent after arecoline 1.5 mg (100.7±6.4 min, 24.7±1.7%) was significantly greater than placebo (76.2±5.4 min, 18.1±0.9%). The data with arecoline, a specific cholinergic muscarinic agonist, confirms our previous findings with physostigmine and suggests that cholinergic stimulation of muscarinic receptors may play a role in the triggering of REM sleep in man. In addition, the results suggest that both arecoline and physostigmine "phase advance" REM sleep towards the onset of sleep; that is, all REM periods occur earlier in the night than under normal conditions.

4. Experimental Shortening of REM Sleep Ultradian Rhythm with Timed Multiple Infusions of Arecoline

Seven normal volunteers (4M, 3F, mean age 25 yrs) were studied under the following two experimental conditions. (1) Pretreatment with methscopolamine 0.5 mg IM followed by three IV infusions of arecoline (1.5 mg, 1.0 mg and 1.0 mg doses) given at the following three times respectively: (a) 35 min after sleep onset; (b) 30 min after end of first REM period; (c) 30 min after end of second REM period. (2) Pretreatment with methscopolamine followed by three placebo (saline) IV infusions. Subjects were run either in pairs or groups of 3 such that the timing of the placebo infusions was "yoked" to the arecoline infusions.

Duration (Minutes) of NREM and REM Periods (Mean ± SEM)

| | NREM ₁ | REM ₁ | NREM ₂ | REM ₂ | NREM ₃ | REM ₃ | NREM ₄ | REM ₄ | NREM ₅ | REM ₅ |
|----------------|-------------------|------------------|-------------------|------------------|-------------------|------------------|-------------------|------------------|-------------------|------------------|
| Placebo | 95.7 +14.7 | 14.1 +3.9 | 89.1 +5.7 | 22.8 +3.2 | 78.4 +4.6 | 25.5 +4.7 | - - | - - | - - | - - |
| Are- coline | 42.2* +2.6 | 10.3 +2.4 | 38.3* +1.3 | 12.8* +2.5 | 41.4* +3.7 | 21.0 +4.9 | 84.7 +4.1 | 22.1 +5.4 | 65.4 +4.6 | 28.8 +5.0 |

(N=6) (N=6)

*p<.01

The results of this study are: (1) It was possible to transform a normal REM ultradian rhythm (with REM-REM interval of about 100 min) into a rhythm with REM₁-REM₂ interval of 48.6 min and REM₂-REM₃ interval of 54.2 min. (2) There were a significantly greater number of REM periods after arecoline (5+0.22) than placebo (3.71+0.29) resulting in greater REM time and percent (arecoline = 104.1+1.5.6 min, 24.8+1.9% and placebo = 84.4+4.6 min, 20.1+1.2%). (3) Duration of individual REM periods was either unchanged or shortened (e.g., REM₂). Acetylcholine plays a role in the timing of REM sleep. The increase in REM time seen appears to result from an increase in the number but not the duration of REM periods. There also appears to be a "ceiling" after which REM time cannot be further increased by cholinergic agents since both single and multiple infusions of arecoline produce the same amount of increase in REM time.

5. Acetylcholine and Sleep: Effect of Oral Choline on Normal Human Sleep

Choline, a normal dietary constituent, has recently been shown to be a precursor of brain acetylcholine. If so, in the light of our previous work with physostigmine and arecoline we would predict that choline administered to humans may alter REM sleep parameters. A double-blind crossover design with 12 normal male subjects was used. Subjects slept for 3 nights (1 adaptation and 2 experimental) and just prior to "lights out" (11:30 p.m.) were given an elixir containing either 10 g of choline chloride or placebo matched for taste, color and consistency.

Effects of Choline Chloride (10 gm) or Placebo

| | <u>Placebo</u> | <u>Choline (10 gm)</u> |
|------------------------|----------------|------------------------|
| Total Recording Period | 472 + 5* | 492 + 4 |
| Sleep Latency | 33 + 8 | 24 + 4 |
| Total Sleep | 422 + 8 | 446 + 6 |
| Non-REM | 326 + 10 | 347 + 8 |
| Stage I | 12 + 3 | 10 + 3 |
| Stage II | 269 + 7 | 273 + 9 |
| Stage III | 22 + 4 | 29 + 4 |
| Stage IV | 25 + 5 | 33 + 5 |
| Stage V | 25 + 5 | 33 + 5 |
| REM Sleep | 95 + 6 | 100 + 5 |
| REM Latency | 92 + 12 | 107 + 12 |
| Intermittent Awake | 13 + 4 | 17 + 5 |
| Early Morning Awake | 4 + 1 | 3 + 1 |

* mean + SEM
values are in minutes

The results were: (1) Choline did not have any effect on conventional sleep parameters when data from all 12 subjects were analyzed. (2) When the subjects were rank ordered with respect to their placebo (baseline) REM latency, REM time and REM %, we found that low placebo REM latencies were increased and high placebo REM latencies were decreased by choline.

The correlation between placebo (baseline) REM latency and the change in REM latency after choline (i.e., choline-placebo value) was an inverse correlation ($r=-0.90$, $N=12$, $p<.01$). Similarly there was an inverse correlation between placebo REM time and REM % and change after choline ($r=0.63$ for REM time and $r=0.61$ for REM%, both $p<.05$). Whether the above changes in REM parameters produced by choline represents a genuine central neuromodulatory effect or is no more than a statistical artifact is unclear. Likewise neither choline (7.5 gm) at 8 am or 11 pm nor choline (7.5 gm) at both 8 am and 11 pm had any effect on sleep as compared with controls. These results suggest choline may not enhance functional cholinergic neurotransmission, at least not in putative sleep centers.

6. The Effect of Piperidine, a Cholinergic Nicotinic Agent on Normal Human Sleep

Piperidine is an alicyclic amine with nicotine-like cholinomimetic effects on central and peripheral nervous system. In view of conflicting reports on the effect of piperidine on REM Sleep in rodents, we undertook to study the effect of oral piperidine HCl in normal volunteers. Using a 4x4 Latin square design, 8 subjects were recorded on 4 nights under the following four experimental conditions (a) placebo, (b) piperidine 200 mg, (c) piperidine 400 mg and (d) piperidine 600 mg.

All medications were given prior to "lights out" (11:30 p.m.). No change in any sleep parameter including REM latency, REM time and REM density, was observed after piperidine. Although data from animal pharmacology suggests that the doses used in our study would have central effects, the possibility still remains that physiologically effective doses were not used. Intravenous administration of piperidine during sleep is also currently underway.

7. Scopolamine-induced Muscarinic Supersensitivity in Normal Man: Changes in Sleep

An injection of scopolamine (6 μ g/kg) was administered on 3 consecutive mornings to normal human subjects. Sleep recordings obtained at night (when the central anticholinergic effect of the morning scopolamine was no longer present) indicated a significant reduction in latency to REM sleep onset on the nights following the second and third injection. REM latency was significantly reduced on the second (50 min + 12) and third (45 min + 13) scopolamine nights as compared with baseline (96 min + 16) ($p<.005$). This effect is opposite to the direct pharmacological action of nighttime administration of scopolamine (i.e., prolongation of REM latency). Furthermore, scopolamine pretreatment on 2 consecutive mornings also potentiated the REM-inducing effect of arecoline, a central muscarinic agonist. The time from infusion to REM was 17 min + 9 after scopolamine pretreatment and 47 min + 11 after saline ($p<.05$). These data are consistent with development of cholinergic supersensitivity following cholinergic blockade.

8. Identification of Stimulatory Cholinergic Mechanisms in Sleep-related Growth Hormone Secretion

We have examined the effects of cholinergic blockade with methscopolamine bromide, 0.5 mg intramuscularly, on sleep-related and insulin-induced growth hormone (GH) and prolactin (PRL) secretion. Thirteen normal young men were studied; eight had sleep studies, and at different times in 3 sleep subjects and in 5 others not studied during sleep, insulin tolerance tests were performed with 0.1 unit/kg insulin. After an adjustment night in the sleep laboratory, saline control and methscopolamine studies were done in a random sequence; study procedures included electroencephalographic, electromyographic, and electro-oculographic recordings, and blood sampling every 20 minutes for hormone radio-immunoassays. On methscopolamine nights, the mean \pm SEM overall GH level of 2.89 ± 0.44 and the mean peak GH level of 11.09 ± 3.11 ng/ml were dramatically reduced to 0.75 ± 0.04 and 1.04 ± 0.25 ng/ml, respectively ($p < 0.0001$ and $p < 0.001$). Despite virtual absence of GH secretion during the night in every study subject, no measured sleep characteristic was significantly affected by methscopolamine, including percentage slow-wave sleep ($12.1 \pm 2.6\%$ control vs. $10.3 \pm 2.5\%$ drug). Sleep PRL concentrations were not changed by methscopolamine. In contrast to the abolition of sleep-related GH secretion, administration of methscopolamine only marginally reduced the GH response to insulin hypoglycemia. Only one of ten time points differed significantly, and the peak concentrations, mean increments, and areas under the curves were not significantly different. We conclude that the burst of GH secretion which normally occurs following sleep onset is primed by a cholinergic mechanism which does not influence slow-wave sleep. Cholinergic mechanisms outside the central nervous system do not appear to play an important role in sleep-related prolactin secretion. The neurotransmitter mechanisms, and presumably the pathways, which subserve sleep-related GH secretion in man appear to be different from those which mediate the GH response to pharmacologic stimuli such as insulin.

9. Piperidine Enhances Sleep-related and Insulin-related Growth Hormone Secretion

Seven male normal adult volunteers slept in the laboratory, and blood samples were drawn every 20 minutes, after the lights were turned out at 11:00 p.m. A 30 minute IV infusion of piperidine or saline was started at sleep onset. In a comparison study, eight other volunteers had blood samples drawn for 2 hours after receiving 0.1 unit/kg regular insulin at 8:00 a.m. A 30 minute infusion of 100 mg piperidine or saline was started 15 minutes before insulin stimulation.

An analysis of variance revealed that piperidine significantly ($p < 0.01$) enhanced sleep-related GH secretion over the 8 hours, from 4.22 ± 0.46 ng/ml on saline to 6.09 ± 0.78 ng/ml on piperidine ($p < 0.02$ by paired t-test). This was particularly striking in the first two hours, when the values rose from 7.18 ± 1.23 to 15.16 ± 2.88 ng/ml ($p < 0.02$). Similarly, there was enhancement of GH secretion during daytime insulin testing. The maximum GH increase after saline was 36.8 ± 3.6 ng/ml compared to 48.0 ± 4.3 ng/ml after piperidine ($p < 0.01$). In order to determine whether the enhanced GH secretion was simply a summation of responses to insulin and piperidine separately,

we also infused piperidine 100 mg alone in seven volunteers. There was no effect on GH concentrations. Prolactin concentrations were also measured during the sleep and insulin studies; there was no significant effect of piperidine.

In sum, this provides further evidence of cholinergic mechanisms in the regulation of these forms of GH secretion, although the relative roles of nicotinic and muscarinic receptors are still unclear. There is little evidence that the cholinergic system plays a significant role in sleep-related or insulin-induced prolactin secretion.

10. Choline Chloride Reduces Insulin-induced Growth Hormone Secretion without Affecting Sleep-related Growth Hormone

Choline chloride (10 gm) or placebo was administered one hour prior to testing the effect of insulin-induced growth hormone release or prior to bedtime. Choline significantly reduced the insulin-induced growth hormone peak from 24 ± 4 to 17 ± 4 ng/ml ($p < .01$). No effect on nocturnal growth hormone or prolactin release was noted. The mechanism by which choline affected insulin-induced growth hormone is not clear, but probably does not result from increased cerebral acetylcholine synthesis.

B. Histamine: Effects of L-Histidine on Sleep

There is considerable indirect evidence suggesting that histamine may function in the brain as a neurohumor in sleep-waking mechanisms. To test the possible role of histamine in the control of waking in man, we administered its amino acid precursor, L-histidine, to human subjects and evaluated their electroencephalographic sleep patterns. L-histidine was given to three patients with intractable narcolepsy (20 gm per day for 2 weeks), to four normal volunteers (32.4 gm per day for 5 days) and to a patient with progressive systemic sclerosis (48.6 gm per day for 16 days). No effects were observed on nocturnal EEG sleep patterns in any of the subjects or on the symptoms of the narcoleptic patients. Although the degree to which histamine levels in the brain were elevated in this study is not clear, these results do not encourage the hypothesis that histamine is an alerting or waking factor in the brain.

C. Catecholamines

1. Differential Effects of D- and L-Amphetamine on the Sleep of Depressed Patients On and Off Lithium Treatment

Dextro (d-) and levo (l-) amphetamine produced different EEG sleep changes in seven depressed patients. Each patient received placebo or one of the isomers (30 mg base) at 7:25 a.m. in a double-blind fashion. The patients were studied under two conditions: without treatment with lithium carbonate and with treatment with lithium carbonate (0.9-2.1 gm/day beginning a minimum of 10 days before study). Both isomers reduced REM sleep and the proportion of total sleep spent in REM (the REM%). No REM rebound was observed on the night following REM suppression. Only d-amphetamine delayed sleep onset and reduced total sleep time, NREM sleep time, and sleep efficiency. The same changes were observed with and without lithium carbonate treatment.

2. Pimozide Attenuates d-Amphetamine-induced Sleep Changes in Man

Pretreatment with pimozide (mean dose=13 mg/day) blocked the effect of d-amphetamine (20 mg base, administered by intravenous bolus infusion at 0815) on all-night EEG sleep patterns in seven hospitalized psychiatric patients. Each patient was studied for five nights (2 nights baseline, 1 night on the day of the infusion, and 2 nights recovery) with and without pretreatment with pimozide. Without treatment with pimozide, d-amphetamine significantly reduced duration of total sleep, REM and nonREM sleep, Stage I, and Stage II. With coadministration of pimozide, d-amphetamine had no effect on sleep. These results suggest that the d-amphetamine-induced changes in sleep are mediated by dopaminergic neurons.

D. Serotonin:

1. Relationship to Narcolepsy

Since serotonin has been implicated in the induction and maintenance of sleep, we administered para-chlorophenylalanine (PCPA 3 gm/day for up to 4 weeks) to 3 patients with narcolepsy. No consistent change in sleep or clinical symptomatology was observed in the 3 patients as a group. One patient, in contrast to our previous experience with PCPA in patients with carcinoid syndrome, actually showed a significant increase in REM while on PCPA, while the other patients showed no decrease in REM. These results suggest (a) that narcolepsy is not related to an abnormality of serotonin and (b) that narcoleptics respond differently to PCPA than carcinoid patients.

2. Reduction of Brain Serotonin and REM Sleep by Acute Administration of a Tryptophan-free Amino Acid Diet

A variety of studies have suggested that the serotonergic system plays an important role in the regulation of sleep. In order to clarify this role, we have performed sleep studies on rats in whom brain serotonin was decreased by a new technique--the acute administration of a tryptophan-free amino acid diet.

Sleep studies were performed on five rats in a paired-data, crossover design. As can be seen on the Table, percentage REM sleep was decreased by one-third ($p < .05$), and there was a small but significant increase in nonREM sleep. Total sleep time was unchanged.

Our observation that an acute reduction in brain serotonin failed to produce decreases in nonREM sleep is in agreement with the results of two studies each in rats and humans. These data seem inconsistent with the hypothesis that the serotonergic system plays an important role in the production of nonREM sleep.

| | Control Diet | Tryptophan-Free Diet | Statistical Significance |
|---------------------------------|-----------------|-------------------------|-----------------------------|
| Total sleep | 519.6+25.7* | 529.8+27.0 | NS |
| Sleep latency | 45.5+10.6* | 35.8+10.6 | NS |
| Intermittent waking (IW/TRP) | 33.9+ 3.0 | 30.8+ 2.2 | NS |
| NonREM sleep (NR/TST) | 87.6+ 2.4 | 91.4+ 2.3 | p<0.05 |
| REM sleep (REM/TST) | 12.3+ 2.4 | 8.5+ 2.2 | p<0.05 |

Values represent mean number of 30 sec. epochs + SEM.

Effects of tryptophan-free diet on brain serotonin and 5HIAA

| Hours after food presentation | Control diet | | Tryptophan-free diet | |
|----------------------------------|--------------|-----------|----------------------|--------------|
| | Serotonin | 5HIAA | Serotonin | 5HIAA |
| 2 | 1.43+0.04 | 0.52+0.06 | 0.80+0.02*** | 0.38+0.02** |
| 4 | | | 0.62+0.01*** | 0.27+0.02*** |
| 8 | | | 0.44+0.02*** | 0.21+0.01*** |
| 24 | 1.52+0.02 | 0.56+0.04 | 0.65+0.02*** | 0.41+0.04* |

Each value represents the mean from four rats + S.E.M., in units of $\mu\text{g/g}$ brain tissue.

* p<0.05 by t-test when compared to mean of combined control values.

** p<0.01

*** p<0.001

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of clinical disorders, biochemistry and sleep.

3. Intravenous Chlorimipramine Affects REM Cycle in Patients with Excessive Daytime Sleepiness and Control Subjects

The sleep of 5 normal volunteers and 4 patients with excessive daytime sleepiness was studied following intravenous administration of 5 mg chlorimipramine during the first period of rapid eye movement (REM) sleep. There was a 95% increase in the duration of the first REM cycle (time lapsed from onset of first REM period to the onset of the second REM period) in all 9 subjects, p<.01, compared to saline control nights. Other effects included a significant, p<.05, mean decrease in the post-infusion REM time and REM percentage to approximately one half of the control levels. Phasic events associated with REM sleep were also diminished during the post-infusion period with significant mean decreases in the REM density and the REM index per minute of sleep.

E. Ethanol:1. A Dose-Response Study of the Acute Effects of Ethanol on the Sleep of Rats

We have administered increasing doses of ethanol (1.1, 1.5, 2.0, 2.5 g/kg) and equivolume saline intraperitoneally to five groups of six rats each. Injections were given five minutes before the beginning of seven-hour recordings, from 9:00 a.m. to 4:00 p.m. The percent of REM decreased and the percent of slow-wave sleep increased. This effect occurred most strongly in the first 3.5 hours of sleep. The number of awakenings were significantly increased in the second half of the recording. This may be a reflection of greatly decreased blood alcohol levels. (A dose of 2.5 g/kg is completely metabolized in about six hours in the rat.) It is of interest that even the highest doses of ethanol did not produce prolongation of total sleep time in either the whole recording or the first or second halves.

2. Hypnotics and Ethanol: Residual EEG Effects

The literature is divided between those who have found a possible synergistic interaction of ethanol and benzodiazepines and those who have not. We have examined this relationship by recording 12-minute waking clinical EEG's in 47 normal young men who received 0.25 mg triazolam, 30 mg flurazepam or placebo, alone or in combination with 0.8 g/kg ethanol. EEG's were performed as a baseline (3:00 p.m.), 50 minutes after receiving drug or drug placebo (5:50 p.m.), 50 minutes after receiving ethanol or ethanol placebo (7:05 p.m.) and 16 hours after having received the drug (9:00 a.m.). Criteria for the presence of drug effects were:

1. Increase in amount and amplitude of sinusoidal rhythmic beta activity.
2. Lower amplitude of background activity.
3. Decrease in amount of alpha activity.
4. Presence of "well-modulated" alpha.
5. Presence of "drug spindles."

Eighty-five percent of the baseline records were correctly identified as having no drug effect. Analysis of the second EEG (50 minutes after drug) and third EEG (50 minutes after ethanol) showed that our interpretations had a significant relationship to whether or not the subject had received the active drug ($p < 0.02$ and $p < 0.05$, respectively). These findings lead us to believe that this method for determining the presence of drug effects is valid.

Analysis of the fourth EEG--16 hours after drug administration--showed no overall difference in incidence of EEG drug effects among the various combinations of drugs and ethanol. Neither drug alone was different from placebo, nor was either drug alone different from itself plus ethanol.

Flurazepam plus alcohol produced drug effects in 86% of the records, compared to 22% in the placebo group ($p < 0.019$; Fisher Exact Probability Test). The difference between incidence of drug effects following

triazolam plus ethanol (56%) and placebo (22%) did not reach statistical significance. There was no difference in frequency of effects between flurazepam plus ethanol and triazolam plus ethanol.

These data lead us to believe that ethanol produces a small but significant increase in the duration of EEG effects of flurazepam. Flurazepam alone is known to remain active for some time (the major metabolite may have a half-life of 50-100 hours). Thus, repeated use of the drug might produce cumulative effects which may possibly be increased by ethanol.

3. Sleep, Behavior, and Blood Ethanol Concentration in the Ethanol-Dependent Rat

In this study sleep recordings were performed to further characterize a previously-described animal model of ethanol dependence. In summary, this consists of administering approximately 10 gm/kg of ethanol per day by intragastric intubation in six divided doses for four days. Eight-hour EEG recordings were made during the baseline period and the first and third days of ethanol administration (induction period). One group of animals (N=14) was also recorded on the first and fourth withdrawal days; a second group (N=5) was recorded on days 1-4 and 6 of withdrawal. During the induction period, REM sleep time was decreased, particularly on the third ethanol day when blood ethanol concentrations were highest.

This largely reflected decreases in the number of REM sleep episodes. The first day of ethanol withdrawal was associated with a decrease in total sleep time, primarily due to loss of nonREM sleep. Total sleep time gradually returned to normal, and in fact slightly exceeded baseline levels on the third withdrawal day. At this time, REM percentage increased to twice the baseline values. This was related to a decrease in REM latency and an increase in the number of REM sleep episodes. REM percentage then decreased such that on the fourth withdrawal day it approached baseline values. Total sleep time and other sleep parameters were also indistinguishable from baseline on the fourth day. Blood ethanol concentrations on the first withdrawal day were inversely related to behavioral scores of withdrawal and to EEG sleep latency.

The major observations in this model seem consistent with observations in the literature on ethanol withdrawal in animals and humans. Several of the EEG parameters--notably the initial decreased sleep and later REM rebound--may be useful in testing new treatments for ethanol dependence.

F. Enzymes: The Relation of Platelet MAO to Human Sleep

We have examined the sleep of normal volunteers with high and low platelet MAO activity. These volunteers were drawn from groups with MAO activity in the highest and lowest decile of a population of 375 students. Ten high (mean 16.1 ± 3.2 [sd] nanomoles per 10^8 platelets per hour) and ten low (7.1 ± 2.3 [sd]) volunteers, 5 men and 5 women in each group returned for sleep studies. Each subject was recorded for two nights following two adjustment nights.

The group with high platelet MAO activity had significantly lower REM sleep time (69.9 ± 5.2 vs 92.7 ± 8.6 minutes; $p < 0.05$), lower REM density 1.5 ± 0.1 vs 1.9 ± 0.1 , and presumably as a result of the previous two factors, a lower REM index, a measure of the total number of eye movements (105.5 ± 9.3 vs 184.8 ± 21.8 ; $p < 0.01$). All other sleep variables were similar for the two groups. These observations constitute the first report relating individual differences in an enzyme involved in neurotransmitter metabolism to human sleep EEG parameters.

G. Arginine Vasotocin Alters REM Sleep in the Rat

Arginine vasotocin (AVT), a pineal peptide, has been reported to inhibit gonadotropin release, modify conditioned behavior, and enhance slow-wave sleep. The latter effect was particularly striking because of the remarkably small doses employed-- 10^{-6} picograms intraventricularly and 1 microgram intraperitoneally in the cat. In order to further characterize the effects of this substance, we are reporting here a dose-response study on sleep and behavior in the rat.

Arginine (8) vasotocin was given intraperitoneally to 200 gm male Sprague Dawley rats in doses of $0.5 \mu\text{g/kg}$ ($n=9$), $50 \mu\text{g/kg}$ ($n=9$) and $500 \mu\text{g/kg}$ ($n=5$), and the EEG and EMG were monitored for 8 hours starting at 8:30 a.m. In a second study, 10 rats received $10 \mu\text{g/kg}$ at 8:30 a.m. and the following behavioral measures were done: righting reflex, an equilibrium test, grasping reflex, catatonia, and tail pinch reflex.

The lowest dose ($0.5 \mu\text{g/kg}$) had no effect on the sleep stages. The middle dose ($50 \mu\text{g/kg}$) decreased REM latency by 65 percent to about 29 minutes ($p < 0.02$) and decreased ($p < 0.008$) REM efficiency from 93.3 ± 1.0 (saline) to 86.9 ± 2.0 (AVT). There was no effect on sleep latency or total sleep. When data were broken down into two hour periods, the major change was a 55% decrease in REM time ($p < 0.02$) in the second two hours. During the first two hours of recording, the animals, when awake, assumed a characteristic quiescent crouching posture, and seemed less responsive to mildly disturbing stimuli such as air blown in the ear. The systematic behavioral tests did not reveal significant changes, although there was a trend toward remaining longer on the equilibrium bar.

At the highest dose ($500 \mu\text{g/kg}$) four out of the five rats died of pulmonary congestion and edema within a few hours.

These data indicate that $50 \mu\text{g/kg}$ has potent physiological effects. Although we did not confirm a report of increased slow-wave sleep in cats, our REM sleep data are reminiscent of the report of decreased REM in the first 5 hours. This also seems to confirm the observation that animals sleeping after AVT administration are easily aroused; when awake they appeared quiescent, yet did not have the neurologic deficits often associated with sedative/hypnotic agents.

H. The Effects of GABA Agonists and Antagonists on Sleep and Behavior in the Rat

It has been estimated that gamma-aminobutyric acid (GABA) functions as a neurotransmitter in 30 percent of mammalian synapses. Given its ubiquitous nature, and recent evidence that some of the effects of benzodiazepine anxiolytics and hypnotics (e.g., the anticonvulsant properties) may be mediated by GABA; it seemed appropriate to carefully assess the effects of GABA agonists and antagonists on EEG measures of sleep. We are reporting the results of administration of 2 and 4 mg/kg of picrotoxin (a GABA antagonist), 82 and 164 mg/kg of imidazole-4-acetic acid or IMA (a GABA agonist) and saline on the sleep and behavior of the rat. All drugs were given at 8:30 a.m. intraperitoneally to 175 gm male Sprague-Dawley rats (approximately 9 in each group), and recordings were performed for 8 hours; because of the relatively short half-lives of these agents, data were also broken down into two-hour segments.

Analysis of the recordings revealed prominent seizure activity in three animals who received the high dose of picrotoxin; they were excluded from the sleep data. An ANOVA demonstrated that among the remaining animals there were significant differences in total sleep ($p < 0.02$) and nonREM sleep ($p < 0.02$) during the first two hours. A Neuman-Keuls analysis revealed that the high dose of IMA increased total sleep to 60.4 ± 8.5 min compared to 23.3 ± 4.0 min for the high dose of picrotoxin ($p < 0.01$). Saline control values for total sleep primarily represented alterations of nonREM sleep.

The effects of these agents on several behavioral tests were assessed in 7 rats in each group. These measures included tail pinch, righting reflex, coordination on a balance bar and catalepsy (time remaining motionless with front feet placed on a horizontal bar). Of these, only the catalepsy test approached significance ($p < 0.06$), indicating prolonged times with the high dose of IMA.

These data seem to confirm previous work suggesting that GABA agonists have sedative qualities. Further studies will be needed to assess the possible role of GABA in natural and pharmacologically-induced sleep.

I. Studies with the Delta Sleep-Inducing Peptide in the Rat.

As part of a series of studies on possible circulating sleep factors, we have examined the effects of a "Sleep Peptide" marketed by Calbiochem (No. 567295), with an amino acid sequence identical to the "delta-sleep-inducing peptide." Originally this material had been discovered in the CSF of rabbits undergoing thalamic stimulation, and was reported to induce delta activity and spindles when administered to rabbits. It was later synthesized and reported to have similar actions. In the studies reported here, the Sleep Peptide or artificial CSF (control) was administered intraventricularly to 200 gm male Sprague-Dawley rats. Drug administration was at 8:30 a.m., under conditions in which lights were on daily from 8:00 a.m. to 8:00 p.m. Recordings lasted 8 hours, and were interpreted in 30 second epochs as waking, non-REM and REM sleep.

Rats were studied with doses of 5.1 $\mu\text{g/kg}$ ($n=11$) and 25.5 $\mu\text{g/kg}$ ($n=10$). When data from the total 8 hour recording with the lower dose were examined, the only significant change was an increase in sleep latency (18.7 ± 4.0 with control injection to 32.7 ± 5.5 min with active drug; $p < 0.009$) and a decrease in nonREM sleep (282.0 ± 13.7 to 249.7 ± 17.6 min; $p < 0.04$). When the first two hours alone were considered, the only significant change was a slight decrease in the number of awakenings (6.4 ± 0.5 to 5.1 ± 0.7 min; $p < 0.05$). At the higher dose, the only significant change was an increase in intermittent waking time (108.4 ± 8.0 to 128.4 ± 7.0 min; $p < 0.039$) over 8 hours, and an increase in REM-nonREM cycle length (14.0 ± 6 to 27.2 ± 5.4 min; $p < 0.02$) in the first 2 hours.

In addition to the sleep studies, a pilot study of the effect of the same 2 doses of the Sleep Peptide on the motor activity of 13 rats was conducted. No gross changes in motor activity were noted.

In summary, intraventricular administration of the Sleep Peptide to rats was found to have only minor effects on the sleep stages, and if anything they were in the direction of slightly disturbed sleep.

J. Lifetime Inhibition of Monoamine Oxidase: Effects on Sleep in the Rat.

Monoamine oxidase (MAO) inhibitors have been widely recognized to be potent suppressors of REM sleep in humans, although most studies have involved inhibitors of both MAO Type A and B, and have been only a few weeks in duration. In this study we have examined the effects of clorgyline, a Type A inhibitor, on the sleep of the rat, both when given subcutaneously and when given over the lifetime of the animal. In the subacute study, clorgyline 2 mg/kg was given daily for 3 days, and an 8 hour sleep recording was performed shortly after the final injection. Biochemical analysis confirmed that MAO-A was significantly ($p < 0.0001$) inhibited by over 90%. It was found that in the 12 rats receiving clorgyline REM sleep time was decreased to 21.5 ± 3.0 min. compared to 33.6 ± 2.7 min. for the 10 control animals ($p < 0.01$). The REM-nonREM cycle length was increased to 39.0 ± 6.2 min. after clorgyline compared to 29.2 ± 2.2 min. for controls ($p < 0.05$).

In the second experiment pregnant rats were implanted with osmotic pumps releasing 1 mg/kg of clorgyline per day, and after birth and weaning the pumps were implanted in the new generation to give a similar dose. When these rats matured, sleep EEG and biochemical studies were performed. Biochemical analysis again revealed that MAO-A was inhibited by over 90% ($p < 0.001$) while MAO-B was unaffected. It was found that despite these marked changes in MAO-A activity the sleep of the 7 clorgyline rats was similar to that of the 7 controls. This may suggest that during chronic MAO inhibition compensatory mechanisms, possibly including altered receptor sensitivity, allow sleep to return to normal.

K. Where is the Hypnotoxin?

It has been hypothesized for many years that a physiologic circulating substance induces sleep and waking behavior, and a number of possible substances have been proposed. In the past no single laboratory has compared

these various materials. For this reason we have examined the effects on EEG-defined sleep in rats of a substance modeled after that of Nagasaki et al.¹, the delta sleep-inducing peptide (DSIP), Factor S derived from the brains of sleep-deprived rabbits, and arginine vasotocin (AVT). Data on the delta sleep-inducing peptide and arginine vasotocin have been presented earlier^{2,3}. Intraventricular administration of 5.1 and 25.5 $\mu\text{g/kg}$ of DSIP was without effect on sleep. Intraperitoneal administration of 50 $\mu\text{g/kg}$ of AVT reduced REM latency and efficiency but did not affect total sleep time or sleep latency. (A lower dose, 0.5 $\mu\text{g/kg}$ was without effect and 500 $\mu\text{g/kg}$ was lethal.) Material from the brains of rats sleep-deprived for 24 hrs.⁴ was derived along the general methods described by Nagasaki et al.¹ The amount derived from the brains of 6 sleep deprived rats was given to each of 10 rats (active group) and that derived from 6 normal rats was given to each of 7 control rats. Other controls included saline (N=10), material which had been boiled (N=9) and material exposed to a protease (N=9). After injections at 8:30 a.m., the only major significant changes were an increase in REM efficiency and decrease in the number of waking episodes during the first 4 hours, but even in these cases the boiled and protease-treated material produced effects similar to the "active" material. A study of intraventricular administration seemed also to suggest very little effectiveness. In another study, Factor S, obtained from Dr. John Pappenheimer, was given intraventricularly over 30 min., starting 8:00 p.m. The dose of Factor S was the amount of material derived from 33 gm of brain tissue. It was found that Factor S significantly prolonged sleep latency (6.2 ± 3.0 min. for control, 46.1 ± 12.1 min. for Factor S; $p < 0.007$), although once asleep there was a reduction in intermittent waking time during the first two hours (66.0 ± 4.0 min. for control, 40.3 ± 6.1 min. for Factor S, $p < 0.01$). Total sleep time was unaffected. Taken together, these data would seem to call for a careful re-evaluation of the existence of currently hypothesized physiologic sleep-promoting substances.

L. Failure of an Inhibitor of Dimethyltryptamine Synthesis to Affect Sleep in Rabbits.

It has been speculated for some time that the conversion of indoles to methylated indoles such as dimethyltryptamine (DMT) may play a role in the genesis of psychotic illnesses. Because of longstanding interest in possible sleep regulating mechanisms involving indoleamines, and reports of altered sleep patterns in schizophrenia, we have examined the effects on sleep of N,N'-Bis-(3-methyl-2-thiazolidinylidene) succinamide, an inhibitor of indoleamine-N-methyltransferase. We gave 68 mg/kg of active drug and saline by acute oral administration at 8:30 a.m. to 9 young male rabbits, and recorded sleep stages for 8 hours. It was found that the agent had no statistically significant effects on sleep.

M. Effects of a Brain Extract from Sleep-deprived Rats on Benzodiazepine Receptor Binding.

The presence in brain of stereospecific binding sites for benzodiazepines raises the possibility that these receptors mediate the therapeutic actions of this important class of sedative/hypnotics. The receptor-binding affinity of various benzodiazepines appears to parallel clinical anxiolytic dosage, potency in the rat conflict test and ability to block metrazol^(R)-induced seizures; the relation to the sleep-inducing

properties of these agents is not yet known. In order to further examine this relationship we have examined the effects of extracts from the brains of sleep-deprived rats on receptor binding in vitro. In each of a series of 5 experiments, 6 rats deprived of sleep for 24 hours and 6 control rats who were allowed to sleep ad lib were killed, and the brains were quickly removed and frozen. The tissue was homogenized in ice-cold buffer (PH 7.4 Tris HCl), and the homogenate was centrifuged at 100,000 g for 60 min. under nitrogen pressure. The resulting supernatant was concentrated with an Amicon PM-10 filter (exclusion limit > 10,000 daltons), and the filtrate was lyophilized and re-constituted in distilled water. Addition of progressively larger amounts of the diazepam crude extract from control rats produced significantly more inhibition of specific H^3 -diazepam binding compared to extract prepared from sleep-deprived rats in four out of five studies. These data suggest that a substance from the brains of sleep-deprived rats may either enhance binding (or reduce inhibition of binding) of benzodiazepines compared to a comparable extract from controls.

N. Local Cerebral Metabolic Activity in Wakefulness and NREM Sleep

Local cerebral glucose utilization has been examined in 74 brain regions with the 2-deoxyglucose method. Mean glucose utilization was lower during NREM in all areas compared with wakefulness. Selected regions are shown (statistically significant in 20 regions) in Table. Units are nmol/100 gm/min.

| | <u>Awake</u> | <u>NREM</u> | <u>% Change</u> | <u>p</u> |
|----------------------------|--------------|-------------|-----------------|----------|
| Striate cortex | 52 + 9 | 34 + 8 | -35 | .054 |
| Inf. parietal cortex | 45 + 5 | 29 + 3 | -35 | .009 |
| Auditory cortex | 82 + 4 | 52 + 11 | -30 | .014 |
| Med. orbital cortex | 53 + 9 | 33 + 3 | -36 | .02 |
| Lat. genic. body | 41 + 7 | 25 + 2 | -39 | .018 |
| Med. genic. body | 73 + 6 | 47 + 3 | -57 | .003 |
| Dorsal med. nuc., thalamus | 52 + 7 | 34 + 4 | -36 | .017 |
| Reticular nuc., thalamus | 29 + 7 | 17 + 2 | -40 | .05 |
| Pulvinar | 43 + 3 | 30 + 4 | -31 | .006 |
| Supra optic nuc. | 29 + 2 | 23 + 3 | -23 | .05 |
| Med. septal nuc. | 28 + 2 | 21 + 2 | -32 | .02 |
| Red nucleus | 52 + 5 | 37 + 2 | -29 | .009 |
| Mes. reticular | 33 + 2 | 22 + 3 | -35 | .003 |
| Dorsal raphe | 36 + 5 | 30 + 4 | -18 | .14 |
| Ventral raphe | 36 + 9 | 29 + 3 | -18 | .28 |
| Sup. colliculus | 55 + 6 | 38 + 4 | -32 | .01 |
| Flocculus | 53 + 8 | 29 + 4 | -46 | .008 |
| Locus ceruleus | 34 + 6 | 29 + 4 | -16 | .25 |
| Corona radiata | 8 + 1 | 4 + 0.1 | -50 | .09 |

The results are consistent with recent studies showing decreased cerebral blood flow during NREM sleep. No metabolically active (NREM) sleep "center" was found. The generalized reduction in brain metabolic activity is consistent with the rest hypothesis of sleep in a limited sense.

II. Hormones:

A. Regulation of Sleep-Related Growth Hormone and Prolactin Secretion

The study of pharmacologic manipulation of sleep-related growth hormone (GH) and prolactin (PRL) secretion has provided data on the roles of the serotonergic, noradrenergic and cholinergic systems in anterior pituitary regulation. The serotonin receptor blocker methysergide (2 mg PO q 6 h) was administered for 48 hours to normal young adults, and blood samples were drawn every 20 minutes during eight-hour sleep recordings. Methysergide administration was associated with an increase in several measures of sleep-related GH secretion. Mean peak concentrations of GH rose from 7.86 ± 1.35 (SEM) ng/ml on control nights to 10.30 ± 1.39 ng/ml with methysergide ($p < 0.03$). Mean nocturnal PRL concentrations were decreased by 70.3%. In contrast to the increases in sleep-related GH secretion, daytime insulin-stimulated GH secretion was decreased 36.4% by methysergide.

These studies would seem to imply that: (1) serotonergic mechanisms may have an inhibitory role, and cholinergic mechanisms may have a facilitory role, in regulation of sleep-related GH secretion; and (2) data drawn from pharmacologic manipulation of daytime insulin provocative testing may lead to erroneous conclusions regarding physiologic control of GH secretion.

B. The Effect of Dexamethasone on Sleep-Related Growth Hormone Secretion in Congenital Adrenal Hyperplasia: Therapeutic Implications

Treatment of children with 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) with steroids has two goals: replacement of inadequate secretion of cortisol (and sometimes aldosterone) and suppression of over-secretion of adrenal androgens. In adults, and adolescents who have achieved full growth, it is most desirable to use long-acting steroids such as dexamethasone (DEX), given at midnight. The effects of DEX on nocturnal GH secretion need to be evaluated, however, particularly before use in children and adolescents who have not achieved full stature. For this reason we have examined GH secretion and other endocrine measures during sleep in CAH patients receiving DEX in two different regimens.

The four females and two males who participated in the study ranged in age from 13 to 22. All but two (who were female) were salt losers. All patients received 0.5 mg DEX for six days each at 0700 and 2300 hours. Sleep studies were performed on night 5, and during a study in which several blood samples were drawn on night 6.

Examination of 24 hour excretion of 17 ketosteroids and pregnanetriol showed adequate suppression under both regimens in four of the six patients. In two subjects there was inadequate suppression with either regimen. LH was found to be secreted episodically throughout the night in a similar and normal manner with both regimens. Mean nocturnal FSH concentrations were also similar.

Examination of sleep-related GH revealed increased secretion during the first two hours of sleep when DEX was given at 0700 compared with administration at 2300. The areas under the curves of GH plots during this

- O. Evidence suggesting that sleep-inducing effects of flurazepam are mediated by benzodiazepine receptors.

Dr. Mendelson, in collaboration with Drs. Skolnick and Paul did dose response studies with 3-hydroxy-B-carboline (3HMC), a selective benzodiazepine receptor antagonist, in rats and showed that the sleep-inducing effects of flurazepam could be blocked. These results suggested that the benzodiazepine receptor may play a physiological role in sleep regulation and in the hypnotic actions of flurazepam, and further, that B-carbolines could be of therapeutic value in disorders of excessive sleepiness.

- P. Stereospecific studies of the benzodiazepine receptor in sleep.

The enantiomers RO-11 6896 (+) and RO 11 6893 (-), high and low affinity benzodiazepine receptor agonists, respectively, were compared in rats. The high affinity isomer reduced sleep latency significantly, whereas the low affinity isomers increased it. The results suggest that high affinity, saturable stereospecific benzodiazepine binding sites play a role in sleep physiology and pharmacology.

- Q. Effect of an adenosine agonist, L-phenylisopropyladenosine (L-PIA)

L-PIA was administered to rats, producing a marked reduction in motor activity, with little effect on EEG defined sleep.

- R. Chelecystokinin: a sleep-inducing peptide?

CCK-8 (10 mg/kg injected intraperitoneally) significantly shortened sleep latency in rats, suggesting that it might be a natural hypnotic.

- S. Inhibition of adenosine deaminase by ENHA produces quiet waking in rats and mice.

An inhibitor of adenosine deaminase, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), produced inhibition of adenosine degradation which temporally paralleled behavioral sedation in both rats and mice.

- T. GABA role in effects of benzodiazepines.

Picrotoxin, an antagonist of GABA, failed to alter the sleep-inducing effects of flurazepam in rats, suggesting that GABA is unlikely to have a role in sleep regulation.

- U. Effect of dipyridamole, an adenosine uptake blocker, on sleep in the rat.

Dipyridamole had little effect on EEG defined sleep.

time were 1737 ± 744 (SEM) and 666 ± 359 ng/ml/min respectively ($p < 0.05$). Following administration at 2300, there was a tendency for increased GH secretion later at night, which did not, however, reach significance.

Only two significant differences were noted on sleep EEG recordings made during the blood study: Following 0700 administration, there was a longer sleep latency (65.8 ± 18.1 vs 24.2 ± 7.9 minutes; $p < 0.02$) and decreased nonREM sleep (266.8 ± 34.4 vs 363.3 ± 13.7 minutes; $p < 0.02$). These observations were not borne out by the recording on night 5, and hence are of questionable biological importance. The possibility remains, of course, that DEX affects sleep differently when patients undergo the slight stress of an intravenous study compared to when they do not.

These observations suggest that administration of DEX at 0700 suppresses the adrenals yet permits sleep-related secretion of GH. Further studies of chronic use, and considerations of possible effects on somatomedins, will be necessary to further establish the safe use of DEX in children and adolescents.

C. LH-RH Administration and Human Sleep.

Luteinizing hormone-releasing hormone (LH-RH), a decapeptide of hypothalamic origin, stimulates release of LH and FSH from the pituitary; in addition it may possess direct actions on the central nervous system. It has been shown, for instance, to cause mating behavior in estrogen-primed ovariectomized rats. Its administration has been reported to produce sexual arousal in human males with hypogonadism well before androgen levels reach the normal range. Because of these implications that LH-RH may possess central effects, and since gonadal hormones have been reported to influence sleep, we have explored the possibility that LH-RH administration may influence normal human sleep.

Subjects were 5 male and 5 female young adult normal volunteers. Females were studied during the follicular phase of the menstrual cycle. Following an adjustment night, each had sleep studies on two nights. On one night they received 100 mg of LH-RH in 500 cc of normal saline intravenously over an 8 hour period starting when the lights were turned out at 11:00 p.m. On the other randomly-sequenced night they received an equivalent volume of normal saline. The dose of LH-RH, while lower than those reported to produce human sexual arousal, has been shown to acutely raise serum LH and FSH.

Sleep EEG data revealed that LH-RH administration produced no change in the total minutes or percentage of the sleep stages, total sleep, or sleep latency. The subjects were also divided into two groups by sex, and once again there were no significant changes in sleep. It is concluded that low doses of LH-RH, sufficient to stimulate gonadal hormones, have no acute effects on normal human sleep. The possibility remains, of course, that higher doses or more potent analogues of LH-RH may have such actions.

D. Effects of Melatonin and Propranolol on Sleep in Rats

Melatonin and L-propranolol, which inhibit melatonin synthesis, were administered to rats at 07.45 h and 19.45 h. Melatonin given in the morning decreased nonREM sleep, but when given at night had no effect on sleep stages. L-propranolol given in the morning had no effect on nonREM sleep, but increased it at night. L-propranolol produced decreases in percentage REM sleep at both times.

E. Changes in Human Sleep Induced by Growth Hormone Administration

Although it has been known for some time that growth hormone (GH) is secreted in relation to sleep, the physiological significance of this phenomenon is unclear. It has been reported in animals that intraperitoneal injections of GH rapidly enter the brain and alter metabolism of biogenic amines, compounds which may play a role in processes such as thermoregulation, memory and sleep. We are reporting here the effects of GH administration on sleep, mood and memory in humans.

Eighteen normal young adult volunteers (16M, 2F) received IM injections of GH and saline 15 minutes before bedtime at 11:00 p.m. and sleep EEG, EOG and EMG were recorded for 8 hours. Doses of GH were 2 (n=8) and 5 (n=10) international units. In a companion study 15 subjects received injections of 2 or 5 units GH at 8:00 a.m., and had the following measures: a mood and behavior scale, 100 mm line measures of alertness and anxiety, serial learning lists, and the Buschke selective reminding test.

The lower dose of GH had no effects on sleep. The higher dose induced a 19% decrease in slow-wave sleep ($p < 0.01$) and a 13% increase in REM sleep ($p < 0.05$). In order to get a sense of GH concentrations related to these changes, serial blood sampling was done in two patients receiving 5 units. It was found that the injections resulted in peak concentrations of 60-80 ng/ml, dropping to less than 10 ng/ml by 7:00 a.m. Other measures, including sleep latency and total sleep, were unaffected. Neither dose, when given in the daytime, affected the mood and memory tests.

This study confirms previous work in animals, indicating that GH administration induces an increase in REM sleep, and also describes a decrease in slow-wave sleep. It raises the possibility that GH secretion may play a role in sleep-stage regulation.

F. Plasma Melatonin Concentrations During Human Sleep

In order to further define the relation of plasma melatonin to sleep, we have studied samples from six normal young adult volunteers (3M, 3F) and analyzed melatonin by means of a new, more sensitive mass spectrometric assay. Samples were drawn every 20 minutes from an indwelling venous catheter from 11:00 p.m. until 7:00 a.m., which coincided with the hours of darkness.

An analysis of variance revealed significant differences between subjects ($p < 0.0001$), different times of the night ($p < 0.0001$) and state of consciousness ($p < 0.009$). Concentrations rose during each of the 3 sequential time periods, progressing from 22.3 ± 4.4 pg/ml in hours 1 and 2, to 38.4 ± 2.8 in hours 3 and 4, to 56.6 ± 2.9 in hours 5 to 8. Analysis of the states of consciousness revealed that samples drawn during intermittent wakefulness were significantly ($p < 0.01$) lower than during the rest of the sleep stages combined, and showed no significant relation to the individual stages.

Previous studies, using radioimmunoassays have indicated either a mean increase at roughly 2:00 a.m. or apparently random peak times; the present work seems to indicate gradual increasing concentrations of melatonin throughout the night. One report indicated no consistent relationship to individual stages of sleep, a finding borne out here. The significantly decreased concentrations during wakefulness, however, suggest that sleep, along with darkness and clock time, may influence melatonin secretion.

G. Effects of Subacute Administration of Growth Hormone on Sleep-related Growth Hormone Secretion.

Previous studies have reported that following repeated administration of growth hormone (GH) there is decreased endogenous GH release in response to insulin. This has been taken to suggest the presence of a negative feedback regulatory mechanism, at least with regard to GH secretion in response to relatively unphysiologically low blood glucose concentrations. We have examined whether repeated injections of GH will diminish the more physiologic sleep-related release of GH. Six young adult normal volunteers were given 2 units of human GH and equivalent saline intramuscularly every 12 hours for a total of 5 injections in a paired-data design study. The final injection was at 5:00 p.m. on the night of the sleep study, so that by bedtime little exogenous GH was circulating, although somatomedin activity was presumably elevated. Sleep EEG data indicated that this procedure had little effect on traditional sleep parameters, most notably no change in delta sleep. On the other hand, an analysis of variance revealed a significant ($p < 0.0001$) effect on the overall night's GH secretion, such that mean plasma concentrations dropped from 4.6 ± 0.7 ng/ml following saline to 2.4 ± 0.2 ng/ml after exogenous GH. There was a significant ($p < 0.0003$) treatment-time interaction, indicating that the decrease was most evident in the first two hours of sleep (10.1 ± 2.3 ng/ml after saline vs. 3.8 ± 0.40 ng/ml after GH). These data seem to suggest that the nervous system is sensitive to the effects of previously high concentrations of circulating GH, and may respond by decreasing sleep-related GH secretion.

H. Effects of Calcitonin on Human Sleep

A previous study has suggested that administration of synthetic salmon calcitonin has daytime tranquilizing or depressant effects in manic or other psychotically agitated patients, yet may reduce nocturnal sleep as determined by 30 minute nurses' sleep checks. This led us to examine the effects of calcitonin on human sleep. Seven young adult normal volunteers

were given skin tests to determine absence of allergy, then were administered 140 MRC units of calcitonin (Calcimar^R) subcutaneously shortly before bedtime. Two subjects subsequently experienced nausea, and their data is not included in this study. Analysis of polygraphic sleep data revealed relatively minor changes in sleep, including an increase in stage 1 (15.2 ± 3.2 min. with saline vs. 28.3 ± 4.2 min. with calcitonin, $p < 0.05$) and a non-significant increase in intermittent waking time (9.4 ± 6.3 vs. 22.7 ± 3.2 min., $p < 0.06$). This seems to suggest that calcitonin may have minor effects on polygraphically-defined sleep, leading perhaps to mild disturbance.

III. Major Findings: Clinical Disorders

A. Successful Separation of Depressed, Normal, and Insomniac Subjects by EEG Sleep Data

Data from all-night EEG sleep studies were used to distinguish normal subjects, primary depressed patients, and primary insomniac patients. In part 1, we compared 41 normal subjects, 56 depressed patients, and 18 insomniacs. In a univariate comparison with normal subjects, depressed patients showed less total sleep, longer sleep latency, more early morning awake time, more intermittent awake time, less delta sleep, less sleep efficiency, and shorter rapid eye movement (REM) latencies; compared with insomniacs, depressed patients showed greater early morning awake time, shorter REM latency, greater REM index, and greater REM density. Using multivariate discriminant analysis, 82% of the sample were correctly classified by diagnosis: 100% of the normal subjects, 72% of the depressed patients, and 77% of the insomniacs. Eight variables contributed to the multivariate separation of depressed individuals from insomniacs and normals: total sleep time, total recording period, sleep efficiency, sleep latency, early morning awake time, awake time, REM time, and REM %. When the discriminant functions were applied to a second group of 18 primary depressed patients, 82% were correctly classified as depressed. These results suggest that primary depressed patients and primary insomniac patients may show relatively characteristic patterns of sleep abnormality.

Part I: Classification of Normal Subjects, Depressed Patients, and Insomniac Patients by Discriminant Analysis: All 12 Sleep Variables

| | <u>Normal</u> | No. (%) <u>Depressed</u> | <u>Insomniacs</u> |
|------------------|---------------|-----------------------------|-------------------|
| Normal (N=41) | 41 (100) | 0 (0) | 0 (0) |
| Depressed (N=56) | 13 (23.2) | 41 (73.2) | 2 (3.6) |
| Insomniac (N=18) | 4 (22.2) | 0 (0) | 14 (77.8) |

B. The "Switch Process" in Manic-depressive Illness: Circadian Variation in Frequency of Switches and Changes in Total Sleep Time.

A "switch" was operationally defined as a rapid change in mood (change of three or more points on a 15-point scale) occurring in less than 24 hours.

Out of 75 bipolar manic-depressive patients, 35 showed 70 switches from depression to mania (D→M) and 37 switches from mania to depression (M→D). Both D→M and M→D switches occur predominantly in the morning (7 a.m.- 3 p.m.). Among the D→M group, patients who switch at night (11 p.m.- 7 a.m.) show significantly greater sleep loss for the next four days (Day 0 to +4), than if they switch in the morning (7 a.m.- 3 p.m.) ($p < 0.01$, Newmann Keuls test). The evening shift does not differ from the other times. The M→D switches, however, fail to show a similar differentiation between the three time shifts with regard to total sleep time. These data indicate that time of switch may predict subsequent sleep changes in D→M switches.

In an unmedicated female bipolar patient fifty-eight all-night polygraphic sleep recordings were obtained during eight switches into and out of mania. While depressed she was hypersomniac and exhibited elevated REM sleep time and short REM latency. On four nights, she apparently switched into mania while asleep. The last recorded sleep stage in each case was REM sleep.

We have analyzed 144 switches (80 switches into mania and 64 out of mania) in another bipolar patient with regular 48-hour manic-depressive cycles. Switches into mania showed a sharp tendency to occur between 8 p.m. and 8 a.m. with peak incidence between 2 a.m. and 4 a.m. Similarly, switches out of mania occurred significantly more often between 4 a.m. and 6 a.m. than during other time periods. The frequency of distribution of these switches into and out of mania was significantly different from an expected random distribution (2XR chi square analysis with $df=11$). Switches during sleep: among the 80 switches into mania 37 occurred out of sleep (41%); among the 64 switches out of mania, 27 were associated with sleep (42%).

C. The Relationship between Changes in REM Sleep and Clinical Improvement in Depressed Patients Treated with Amitriptyline

EEG sleep recordings were obtained on consecutive nights from six hospitalized depressed patients before, during, and after treatment with amitriptyline. Amitriptyline significantly reduced time spent in Rapid Eye Movement (REM) Sleep and prolonged the REM Latency throughout the treatment period. Three patients who improved during treatment had a REM rebound when amitriptyline was discontinued, whereas three patients who did not improve showed no REM rebound.

D. Muscarinic Supersensitivity: A Possible Model for the Sleep Disturbance of Primary Depression

The sleep changes induced in eleven normal volunteers following the administration of scopolamine on 3 consecutive mornings resembles many of the abnormalities observed in the sleep of patients with primary depression: increased sleep latency, and reduced REM latency, total sleep time, and sleep efficiency. Furthermore, multivariate discriminant analysis, previously shown to distinguish the sleep records of depressed patients from those of normal controls and insomniac patients, selected the records from baseline nights as normal and those after scopolamine as predominantly depressed.

| | <u>Baseline</u> | <u>Scopolamine</u> |
|------------------------|-----------------|--------------------|
| Normal | 11 | 4 |
| Depressed or Insomniac | 0 | 7* |

$p < .005$ Fishers Exact Probability Test

* depressed 6
insomniac 1

These observations suggest that muscarinic supersensitivity in normals may function as a pharmacological model for the sleep disturbances of depression.

E. Faster Cholinergic REM Induction in Remitted Patients with Primary Affective Illness

We compared remitted patients with primary affective illness and normal control subjects on the "cholinergic REM-induction test." In this test we measure the speed with which REM sleep is induced by arecoline, a cholinergic muscarinic agonist administered intravenously during the second nonREM sleep period.

The control subjects were sixteen paid normal volunteers (9 males, 7 females, mean \pm SD, age = 28.3 ± 5.4 years). They were compared with two groups of remitted patients with primary affective illness. The initial group (Group I) of thirteen remitted patients (4 males, 9 females, 12 bipolar and 1 unipolar, mean age 28.9 ± 6.9 years) was tested after all their regular medications had been discontinued for at least 2 weeks. To rule out potential confounding effects of prior psychoactive drug treatment or withdrawal on the arecoline response of Group I, we recruited a second group (Group II) of eight remitted patients who had never received any somatic therapy (4 patients) or had not received it within 4 months of the study (4 patients). The second group consisted of 6 bipolar and 2 unipolar affective patients (3 males, 5 females, mean \pm SD, age = 29.1 ± 5.6 years). All patients met research diagnostic criteria for primary affective illness and had been in clinical remission for at least 3 months at the time of the study.

Arecoline induced REM sleep significantly faster in both patient groups than in controls. Following arecoline 0.5 mg, mean Inf-REM₂ latency was 38 min in normals, 11 min in Group I, and 15 min in Group II ($p < .01$, $H = 10.22$, $df = 2$, Kruskal-Wallis one-way analysis of variance). The mean Inf-REM₂ duration was significantly shorter for both Group I ($p < .01$) and II ($p < .025$, Mann-Whitney U-test) than for normal controls. Arecoline 1.0 mg was administered only to normal controls and Group I; again, the patients entered REM significantly faster than controls (7 min versus 9 min, $p < .01$, Mann-Whitney U-test).

F. Genetic Influences on Response to Cholinergic REM Induction Test.

Seven pairs of identical twins were studied. Results indicate that the response was significantly influenced by genetic factors. These results suggest that the Cholinergic REM Induction Test measures a state independent genetic marker for affective illness.

G. Catecholamine Depletion: Sleep and Mood Changes in Man Mimic the Bipolar Switch Process

Alpha-methyl-para-tyrosine (AMPT, 3 gm/d) was administered in a double-blind fashion to 9 normal volunteers (mean age 24) for 3 days. During AMPT administration, some ratings of mood indicated significantly increased depression, while sleep studies indicated hypersomnia and reduced REM sleep. During withdrawal from AMPT mood ratings suggested hypomania and sleep studies indicated a transient hyposomnia. Co-administration of lithium (900-1200 mg/day) attenuated the withdrawal hypomania. These results suggest that AMPT in normals mimics the bipolar "switch" process from depression to mania.

H. Sleep of Bipolar and Unipolar Depressed Patients Compared

The sleep of 36 normals, 36 unipolar patients, and 22 bipolar patients was compared. The only sleep variable which significantly differed between unipolar and bipolar patients was REM efficiency. Bipolar patients showed a significant reduction compared with unipolar patients ($82\% \pm 3$ versus $91\% \pm 1$, $p < .001$). In addition, "hypersomnia" (defined as more sleep than the age-corrected normal mean) was more prevalent among the bipolars than the unipolars ($p < .05$, $\chi^2 = 3.81$).

I. Schizophrenia

1. Schizophrenia:

Eight actively ill schizophrenics and eight nonpsychotic controls were deprived of REM sleep by the awakening method for two nights. Sleep patterns during five post-deprivation nights were analyzed by a variety of multivariate techniques.

Data suggest that actively ill schizophrenics are less likely than control psychiatric patients to exhibit a normal REM rebound. They require fewer awakenings than controls to achieve REM deprivation. They show little or no change in REM time or REM% during recovery as compared with baseline, and, compared with controls, have significantly less REM time, REM%, and change in REM time and REM% on early post-deprivation nights.

The two patient groups also differed in their pattern of stages III and IV during recovery. Considerable overlap existed in REM compensation between actively ill schizophrenics and controls. Additional information suggests that REM compensation may be related to Rod and Frame testing: the more field independent a subject is, the better REM compensator he is.

2. 5-Hydroxytryptophan and Schizophrenia

5-Hydroxytryptophan was studied at doses of 200-60000 mg/24 hr with MK486 in 18 chronic schizophrenic patients. EEG sleep studies revealed:

(a) REM sleep was reduced at all doses; no rebound was found with 5HTP withdrawal; (b) REM latency was increased at all doses; and (c) NREM sleep was increased at low doses, decreased at high doses, and decreased still further during withdrawal.

J. Sleep in Gilles de la Tourette's Syndrome

The sleep of 6 Tourette patients (drug-free and while taking haloperidol) was compared with 9 normal volunteers. The untreated patients had 30% less delta sleep, which returned to control values when they received haloperidol.

K. A Case of Sleep-walking and Somnambulistic Homicide Associated with Psychotropic Drugs

The patient is a 44 year-old white female who had a history of one sleep-walking episode in adolescence but none after that until age 40. She also had had enuresis until age 16. Except for brief psychiatric treatment at age 16 for enuresis and anxiety, she had no psychiatric history until age 39, when she developed a florid psychosis with persecutory symptoms. She was diagnosed as schizophrenic, hospitalized for 2 weeks, and treated with phenothiazines. A year later she experienced a return of symptoms and resumed treatment on an outpatient basis. Because of insomnia, she received thioridazine and Triclos (a chloral derivative) at bedtime. At 3 a.m. on the third night of this regimen, she arose from her bed and stabbed her daughter to death, possibly while asleep or in a confused state. She was found to be hallucinating and delusional when examined the next morning and diagnosed as suffering from paranoid schizophrenia. At her trial she was found innocent on the grounds of insanity and hospitalized under court order.

On phenothiazines and psychotherapy, her psychosis slowly cleared. However, she was noted to sleep-walk 2-4 times per week. Physical investigations, which eventually included EEG's with nasal-pharyngeal leads, a CAT scan, and a pneumoencephalogram, were all normal. Dilantin and tricyclic antidepressants had no effect on her sleep-walking.

After 4 years in the hospital, she was transferred to our unit, and thioridazine 300 mg q.h.s. was discontinued. During a one-month drug-free period, 11 all-night sleep EEG's along with continuous video monitoring were carried out. There were no sleep-walking episodes. However, when treated with thioridazine 300 mg q.h.s. and chloral hydrate 1000 mg q.h.s. she sleep-walked on 5 of the 6 nights recorded. On thioridazine 300 mg q.h.s. alone, she walked one night in four; on chloral hydrate 2000 mg alone, she did not walk during three nights of recording. All sleep-walking episodes occurred out of slow-wave sleep.

These results suggest that she sleep-walked only on psychotropic medications, particularly thioridazine. This case is similar to those reported by Huapaya (Am. J. Psychiatry 133: 1207, 1976) of somnambulism secondary to bedtime doses of methaqualone in two cases, chlorprothixene in another, and the combination of methaqualone and thioridazine in the fourth. In all

cases sleep-walking could be abolished by either reducing or eliminating the bedtime dose. Two of the cases had childhood histories of sleep-walking, as did our own. As well, Flemenbaum (Am. J. Psychiatry 133:570-572, 1976) has reported cases of pavor nocturnus, another disorder associated with stage 4 sleep in patients receiving large bedtime doses of tricyclics or antipsychotics. The mechanism of this phenomenon, which may not be uncommon, is unknown. We are presently analyzing our patients' sleep EEG's.

Because of continued interest in issues of efficacy and safety of long-term hypnotic administration, we have performed a study of EEG-defined sleep, subjective reports of sleep, and daytime functioning in 10 insomniacs. These subjects were chosen on the basis of a report of chronic sleep disturbance and the absence of medical, psychiatric, or diagnosed sleep-related pathology.

The major EEG effects of flurazepam (30 mg HS for 28 nights) were an increase in total sleep time during the period of days 13, 15, and 27, 28 ($p < 0.05$) but no significant decrease in sleep latency or intermittent waking time. REM percentage was significantly decreased ($p < 0.05$), particularly on days 13 and 15, as was percentage stage 4 ($p < 0.05$) on days 27 and 28. Subjective reports of sleep indicated improvements in quality of sleep, difficulty getting to sleep, and sleep latency, primarily in the first week of treatment. There was a non-significant increase in perception of total sleep time. Blood concentrations of desalkylflurazepam rose from the general range of 18.1-62.4 ng/ml on the morning after one dose to 41.2-169.4 on day 13 to 41.0-179.9 on day 26. In the daytime there was no significant alteration in sleepiness, activation, dysphoria or depression, although there were significant decreases in the euphoria scale ("sense of well-being") during chronic drug administration and withdrawal. Daytime testing showed marked impairment of time to do letter cancellation test and a variety of cognitive tasks, including serial learning, prompted recall and free recall tasks. A questionnaire of physical symptoms showed no major changes in the 10 subjects. A single subject, who had mild respiratory changes before the study, developed a sleep apnea syndrome by the second night of flurazepam, which cleared upon cessation of the drug.

L. Flurazepam-induced Sleep Apnea Syndrome in an Insomniac.

Sleep, EEG, and respiratory measures were examined in a 38-year-old man with a long-standing history of insomnia and daytime sleepiness. He was found to have seven to 18 primarily obstructive apneas per night on four baseline recordings, a finding not generally considered to be indicative of pathology. On the first two nights on which he received 30 mg of the benzodiazepine hypnotic flurazepam, there were 22 and 100 apneas, and during the daytime he became extremely sleepy. Upon cessation of medication, his clinical condition improved, and the number of apneas decreased to 11 and 6 on withdrawal nights 4 and 6. Although respiratory depression is neither invariable nor unique to flurazepam, this case suggests that it may be a clinically significant problem with recommended oral doses in some individuals.

M. Sleep Abnormalities in Obsessive-Compulsives.

EEG abnormalities of both adolescents and adults with obsessive-compulsive disorder reveal sleep abnormalities similar to those seen in patients with primary or endogenous depression, i.e., short REM Latency and Total Sleep Time.

These findings challenge the hypothesis that short REM Latency is a biological marker of primary or endogenous affective illness. They suggest the sleep disturbances of primary or endogenous depressions are sensitive but not necessarily specific for the diagnosis.

N. Nap Studies in Affective Illness.

Since no collaborative investigators within the Intramural Research Program were interested in using nap studies in depressed patients to test the circadian phase-advance hypothesis or over-arousal hypothesis, we collaborated with Dr. David Kupfer and his group. Patients were encouraged to nap at 10 am or 3 pm on successive days. The patients appeared to take longer than normal (literature reviews) to fall asleep, especially in the afternoon. Furthermore, of those who did have REM sleep in the daytime naps, REM Latency was similar in the morning, afternoon, and evening. This is in contrast to what has been reported in normals. Nevertheless, REM propensity was highest in the morning in all the patients, as has been reported in normals. In addition, patients who responded to tricyclic therapy later on were found to sleep more poorly during the naps than non-responders.

These results do not offer support for either the free-running or the phase-advance hypothesis of depression but do suggest that circadian disturbances of sleep propensity could be of significance. Because of the unexpected difference between responders and nonresponders in their napping propensity (but not their nocturnal sleep), further studies would be of great interest to both theoretical and clinical response issues. In any case, such studies combined with other measures, such as temperature and cortisol, offer fast and inexpensive ways of testing hypotheses linking affective illness and biological rhythms.

O. Cross-Site Validation of Sleep Disturbances in Affective Illness.

In a collaborative study with Michael Feinberg and Bernie Carroll of the University of Michigan, sleep data from the NIMH and from Ann Arbor were compared and found to be remarkably similar for patients with endogenous depression. Furthermore, discriminant analysis, based on one set of data, identified patients from the other set with remarkable sensitivity and specificity. This is one of the few instances in biological psychiatry where the measures in one laboratory can be used to identify patients in an entirely different laboratory. They reinforce the hypothesis that patients with endogenous affective illness share common biological abnormalities. Of the various biological measures in affective illness, sleep appears to offer the greatest sensitivity.

P. Age-related Changes in Sleep in Normals and Depressives.

Both patients with depression and normals showed age-related reductions in total delta sleep, REM latency, and sleep efficiency, but patients tended to show greater disturbances of sleep continuity with age than normals, particularly in early morning awakening. The results provided limited support for the hypothesis that the sleep of depressives represents accelerated aging.

IV. Major Findings: Normal Studies

A. Effects of Sleep Stage and Stimulus Intensity on Auditory Evoked Responses

The purpose of this study was to evaluate the effect of sleep stage and stimulus intensity on the auditory evoked response (AER). Nine normal volunteers (age 18-22; 6M, 3F) slept in the sleep lab for three nights each; the first night was adaptation and was not included in any calculations. Four intensities of clicks (50, 60, 70 and 80 dB) were generated by the LINC computer in pseudo random order. EEG was sampled at 250 Hz for 1 second and AER was computed on line for each intensity for each minute of the night. Each minute was visually scored for sleep stage. AER from the minutes of each sleep stage were then averaged. AER amplitude tended to increase little from 50 to 80 dB in waking subjects but increased markedly in sleeping subjects in stage 3 and 4 ($p < 0.05$, 2-way analysis of variance). REM and stage I sleep had small amplitude AER's in comparison with other sleep stages.

Individuals who showed decreases in amplitude at high intensities while awake slept significantly longer during the experimental night ($r = .76$, $p < 0.05$). The loss of inhibitory mechanisms during stages 3 and 4 is well known. It is therefore not surprising to see the disappearance of the paradoxical AER amplitude reduction at high intensities and a more linear EP amplitude/stimulus intensity function; stage 1, 2, and REM sleep resembled the waking state. Of interest was the finding that waking AER amplitude/stimulus intensity functions predicted sleep duration in this noisy environment. This suggests that individual differences in sleep duration may be related to differences in inhibition of sensory processing.

B. EEG Cortical Map in Sleep

Computer-generated cortical maps of power spectral estimates derived from 16 leads were drawn based on daytime sleep recordings in four normal epochs from awake, stages 1-4, and REM sleep in each volunteer. EEG leads were placed on the left hemisphere and midline according to the 10-20 system with four additional interpolated posterior locations. Spectral estimates with 1 Hz resolution and several frequency bands (delta 2-4; alpha 8-12, beta 13-18) were analyzed with a two-way ANOVA (lead by sleep stage). Delta activity was relatively uniform and of low amplitude in awake, eyes-closed subjects and REM. Delta power increases at the vertex in stage 1 which, with successive NREM sleep stages, both progresses in power and enlarges radially to the intraparietal sulcus posteriorly and the superior frontal gyrus anteriorly. Beta activity in awake subjects was low and maximal parietally. Stages 1 and REM showed even lower and more uniform distribution. Stage 2 showed the greatest power, concentrated at the vertex, with stages 3 and 4 diminishing. Alpha activity was expectedly maximal occipitally in awake subjects, but surprisingly a frontal area appears in slow-wave sleep. All findings were confirmed by a two-way interaction. These data indicate that the sleep stages are not electrophysiologically uniform across the cortex and open the possibility for a new approach to the diagnosis of sleep and psychiatric disorders.

V. Major Findings: Animal Behavior

A. Deprivation of REM Sleep and Nesting Behavior in Rats

The effects of REM deprivation for four days on nesting and locomotor activities were studied in male rats. Four groups of animals were tested: (a) Experimental animals (SP), deprived of REM on a small (6.5 cm) pot; (b) large pot (LP) control, maintained on large (12.5 cm) pots; (c) stress group, forced swimming for 1 hr/day; (d) normal controls. The results indicate, as predicted, increased utilization of nesting material by the experimental (SP) group in the post-REM deprivation period as compared with normal controls (464 cm for SP, 94 for normals, $p < .05$). Nevertheless, the SP group did not differ significantly from LP (356 cm) or stress (133 cm). Since LP does suffer some REM deprivation, though less than SP, the results suggest that increasing levels of REM deprivation may increase a drive-related behavior in rats.

B. EEG Documentation of a Method for Sleep Depriving Rats

There is a need for a relatively simple method of sleep deprivation which is supported by EEG data and which can process a number of animals at once with a minimum of labor. We shall describe such a technique, which consists of keeping rats awake by maintaining them in slowly rotating cylinders.

Rats were placed in two Nalgene cylinders (68.6 x 35.6 cm), each of which is divided into six compartments. An electric motor causes them to rotate at 3 RPM. A strip of tubing on the floor of each compartment provides an obstruction over which rats must walk, so that they cannot slide along.

In order to document the state of consciousness of rats undergoing this procedure, we recorded the EEG and EMG of four rats in the moving cylinders for 24 hours and compared them to seven animals in a stationary cage. The moving cylinder reduced sleeping time from 47.0% of the 24-hour period to only 3.8% ($p < 0.001$). The small amount of sleep which did occur was made up of both REM and NREM sleep. The percentages of total sleep time did not differ between the two conditions. Thus, there was no selective effect on one particular sleep stage, and instead total sleep time as a whole was markedly reduced.

C. A Signal Analysis of the Rat Sleep EEG: The Application of Continuous Frequency Analysis

Automated rat sleep analysis focuses on the statistically regular waveforms of the EEG, such as theta and delta rhythms. Such stochastic processes can be quantified in several manners. Time domain statistics such as auto- and cross-correlations produce outputs that are difficult to use and are best performed in software. Frequency domain statistics like spectral density accurately quantify the sleep state by power

frequency distributions but also require sophisticated computer processing. Continuous frequency analysis, using passband filtering, accurately measures signal power in an on-line fashion and employs relatively inexpensive hardware to estimate power by integrating the square of the signal. This method differs substantively from other previously reported systems which rely on signal amplitude analysis. Comparison of this system with a human scorer indicates high degrees of validity and reproducibility.

D. Sleep in Rats with Bilateral Habenular Lesions

The diencephalic habenula nuclei (HBN), which receive afferents from several limbic structures, are thought to send an inhibitory pathway to the dorsal raphe nuclei (DRN). Thus electrical stimulation of the HBN has been reported to decrease DRN cell firing, and bilateral lesions of the HBN may lead to a 60% increase in HIAA content of the DRN (manuscript in preparation). These lesions have been associated with increased daytime motor activity in rats (manuscript in preparation). Because of longstanding interest in the role of the raphe in sleep regulation, we have examined the effect of bilateral HBN lesions on sleep in the rat.

Ten male 200 gm Sprague Dawley rats received bilateral radio frequency lesions of the HBN, and 11 had sham operations. They were then implanted with EEG and EMG electrodes, and 8 hour recordings starting at 8:30 a.m. were performed one week later. Analysis of the data revealed no significant changes in sleep latency, total sleep, or any sleep stage measure.

A lesion producing relatively drastic changes in dorsal raphe cell activity might well be expected to influence sleep, and it is thus surprising that no such change was found. Alternative explanations for this might be that (1) lesion effects are seen only at other parts of the circadian cycle; or (2) during the week between lesioning and recording, the nervous system develops tolerance to perturbations from altered serotonin metabolism. One argument in favor of the latter hypothesis comes from reports of tolerance to the effects on sleep of the serotonin synthesis inhibitor parachlorophenylalanine.

VI. Studies of Human Memory:

A. Effects of Arecoline or Scopolamine Prior to Learning.

We determined the effects of arecoline and scopolamine on categorized serial learning in normal volunteers, all of whom were college students or recent college graduates.

The results indicated that arecoline (4 mg) significantly reduced the number of trials to criterion as compared with saline (mean 3.76 vs. 5.18 trials, $p < .01$). In contrast, the mean number of trials following scopolamine (0.5 mg) was 6.35 ($p < .01$, compared with 5.18 on methscopolamine-saline control). Administration of arecoline 6 mg, however,

reversed the effects of scopolamine 0.5 mg (mean number of trials was 4.92, which was significant, $p < .01$, compared with scopolamine alone).

We also found that the major effects of arecoline and scopolamine were seen in naturally "poor" performers rather than "good" performers. The correlation between baseline, placebo performance and change on arecoline was $r = 0.93$, $p < .001$, indicating that greater improvement was seen in those subjects who took the most trials to criterion on baseline; for scopolamine, the correlation was $r = 0.5$, $p < .05$, indicating that the greatest decrement in performance was seen in those who took the most trials to criterion.

B. Effects of Arecoline Following Learning

We showed that administration of arecoline immediately after learning facilitates later recall. Eight subjects (5 males, 3 females, mean age 24) were tested on two nonconsecutive days. Prior to the experiment, an intravenous needle was inserted in a forearm vein and attached to a ten-foot catheter extending out of the room so that drugs could be administered without the awareness of the subject. Following administration of methscopolamine (0.3 mg iv), subjects heard a list of words, nine groups of five words each. Three of the nine groups consisted of highly related (associated) words, three groups of weakly associated words, and three groups of randomly associated words. The groups were presented in order: first a highly related group, then a weakly associated group, then a randomly associated group, until all nine groups had been presented. After listening to each group of five words, subjects were asked to think of and say a word which defined or organized that word cluster. Immediately at the end of the session, subjects received a twenty minute infusion of either arecoline (2 mg in 20 ml of saline) or placebo (20 ml saline). Recall testing began 1 hour following the onset of the infusion. Subjects were tested first under free recall conditions and then under "cued" recall conditions (after having been presented with their own, self-generated cue word).

Compared with placebo infusion, arecoline significantly improved recall of the presented word; all three types of word sets--highly associated, weakly associated, and randomly associated--were better recalled ($p < .05$, $F = 7.8$, $df = 1,7$). This was true under both free and cued recall conditions. Interestingly, the arecoline effect appeared to be mediated by an increase in the number of clusters recalled ($p < .01$, $F = 13.6$, $df = 1,7$) rather than an increase in the number of words per cluster. While cued recall was better than free recall ($p < .001$, $t = 29.6$, $df = 1,7$), and highly associated word sets were better recalled than weakly or randomly associated words, the facilitory effect of arecoline did not interact with these factors.

C. Effects of Choline Chloride on Human Learning

To study the effects of choline chloride in man, we administered an elixir containing 10 gm or placebo on separate days. Ninety minutes later, we began the session with two different tests: uncategorized serial learning and a selective reminding task.

Choline administration improved performance on both tests. On the uncategorized serial learning tasks, subjects learned to recite ten unrelated words in correct order; following each trial, the entire list was repeated by the experimenter. The number of trials to criterion was 5.7 ± 0.7 after choline and 6.1 ± 0.9 after placebo ($p < .05$, two-tailed t-test). On the selective reminding task, subjects learned a 12 word list of words, half of which were high image words (such as table) and half of which were low image words (such as virtue). Following each trial, the investigator reminded the subject of the words missed on the last trial, but did not repeat the words correctly remembered. As might be expected, high imagery words were learned in fewer trials on placebo than low imagery words, and choline did not alter the number of trials required for high imagery words. Choline administration, however, significantly reduced the number of trials required to learn low imagery words from 9.1 ± 0.9 to 6.8 ± 0.5 ($p < .01$).

The effects of choline were significantly correlated with performance on placebo, indicating a greater effect in "poor" performers than "good" performers. The change induced by choline was significantly correlated with performance on placebo on the uncategorized serial learning task ($r = 0.59$, $p < .05$, $N = 10$).

D. Effects of Lecithin on Memory and Plasma Choline Levels in Normal Volunteers.

A pure grade of lecithin (phospholipon 100 - about 95% phosphatidylcholine) was administered to 15 normal volunteers in doses of 20, 40, and 60 gm. A dose-dependent elevation of plasma choline was noted, which persisted for at least 23 hours. The highest dose produced some nausea and GI symptoms. No overall change in memory and learning was noted. The "poor performers," however, again showed a significant dose-dependent increase in performance at 20 and 40 gm as compared with placebo.

E. Cholinergic Therapies in Alzheimer's Disease

Preliminary data fails to show a significant effect of lecithin in patients with clinically defined Alzheimer's disease. Nevertheless, it does appear that the combination of lecithin and THA (an oral anticholinesterase) may improve cognitive performance. Further studies are underway.

VIII. Thermoregulation in Patients with Primary Affective Illness

The regulation of body temperature may be another CNS function which may be under the partial control of acetylcholine. We studied the hypothermic response of arecoline in a group of 17 normals and 10 primary affective patients in complete remission who were drug free for more than 2 weeks. Arecoline was administered as a slow IV drip (.066 mg per min, total dose - 2 mg every 10 min). There was no difference between normals and patients with respect to the hypothermic effect of arecoline.

Our data suggests that patients with primary affective illness appear to be hyper-responsive to pharmacological challenge with cholinergic agonists in two out of three measures tested; i.e., REM sleep induction and pupillary miosis. They did not differ from normals with respect to the hypothermic effect of arecoline. The regulation of temperature, like blood pressure, is, of course, a very complex central and peripheral nervous system function. Thus, it may not be an appropriate physiological system to measure specific cholinergic effects.

Our aim has been to develop pharmacological challenge strategies utilizing physiological endpoints that could be objectively measured. Our data strongly supports the existence of a hyper-responsive cholinergic system in affective illness, both during the depressed and euthymic (remitted) states.

IX. Seasonal Affective Illness and the Antidepressant Effects of Light.

Dr. Normal Rosenthal of this Unit, in collaboration with Drs. Wehr and Sack, has identified a group of subjects who experience regular depression in the fall-winter and hypomania in the spring-fall. The severity of affective illness is rarely severe enough to warrant major psychiatric intervention, but is nonetheless disabling and troublesome. Interestingly, these patients experience hypersomnia and hyperphagia during depression in contrast to insomnia and anorexia typically seen in most depressives.

In a prospective study of 24 subjects with seasonal mood disorder (as described above), 11 were eventually included in a study of the effects of light therapy on winter depression. Subjects were assigned to either bright lights or dim lights for an additional six hours of light exposure per 24 hours (i.e. from 4 to 7 am and from 6 to 9 pm). Bright lights proved to have a significant antidepressant effect (Hamilton Score, $p < .001$) whereas dim lights did not. Sleep studies indicated a significant increase in both REM and NREM sleep, but a reduction in Delta Sleep, from summer to winter.

X. Significance to Biomedical Research and Proposed Course of Project

This annual report marks the end of a relatively strong and independent human research program within the Intramural Program. These studies were initiated by Frederick Snyder in the early 1960's at the suggestion of David Hamburg, and have been advanced at various times by Allan Hobson, Ernest Hartmann, Ismet Karacan, David Kupfer, Richard Wyatt, Dennis Murphy, Robert Post and others, including some still associated with the Unit on Sleep Studies. Findings from these studies have contributed greatly to our understanding of basic sleep-wake mechanisms, and of the biological basis of psychiatric illness, certain medical illnesses, and sleep disorders. They have also had an impact on neuropharmacology, neurophysiology, neuroendocrinology, psychiatric diagnosis and treatment. It is probably no exaggeration to say that the clinical studies initiated here during the 1960's and carried on here and elsewhere since then have led to the best established and most sensitive biological findings in affective illness and have provided the empirical basis for many current theories of affective illness, such as the chronobiological perspectives (i.e., the circadian phase advance

hypothesis) and various neurochemical hypotheses. Findings in recent years in this laboratory have led to the hypothesis that patients with major affective illness have a genetically inherited, state-independent cholinergic supersensitivity, a finding which Dr. Gershon and his collaborators have tentatively confirmed with biochemical measurements of muscarinic receptors in fibroblasts from patients and their relatives. In addition, recent studies have established an important role for the benzodiazepine receptor in normal and pharmacological sleep mechanisms.

At the present time, support for a strong Unit on Sleep Studies has been discontinued in order to provide personnel for the new chronobiology laboratory. It is not, however, too early to speculate that the IRP will ultimately see the need to support a strong sleep program. Why?

(1) Clinical research will need a strong sleep program.

It is inconceivable that a strong program of research in affective illness or chronobiology can be at the forefront without it.

(2) Clinical needs will require a strong sleep program.

Sleep related disorders are common, and a great deal has been learned recently, leading to a heightened interest and need to provide these services to the Clinical Center and to patients in general. Already we receive about two requests a month to evaluate patients in other institutes for sleep apnea, excessive daytime sleepiness, or insomnia. We have tried to provide the best consultation possible with limited resources, but eventually the Clinical Center will need this clinical capability if it is to be a great medical center. There will continue to be considerable areas of scientific collaboration with other institutes.

(3) Political and administrative needs will demand a strong sleep program.

Public and scientific interest in sleep disorders is great, and there is general recognition that much more research in these areas is needed for the public good. Several years ago, for example, the National Academy of Sciences recommended a greater federal effort in these areas.

(4) Intellectual curiosity will require a strong sleep program.

Persons of intellectual curiosity and vision will always rest uneasily until the "how's" and the "why's" of the sleep-wake states are known. "You spend a third of your life asleep; you really should learn more about it."

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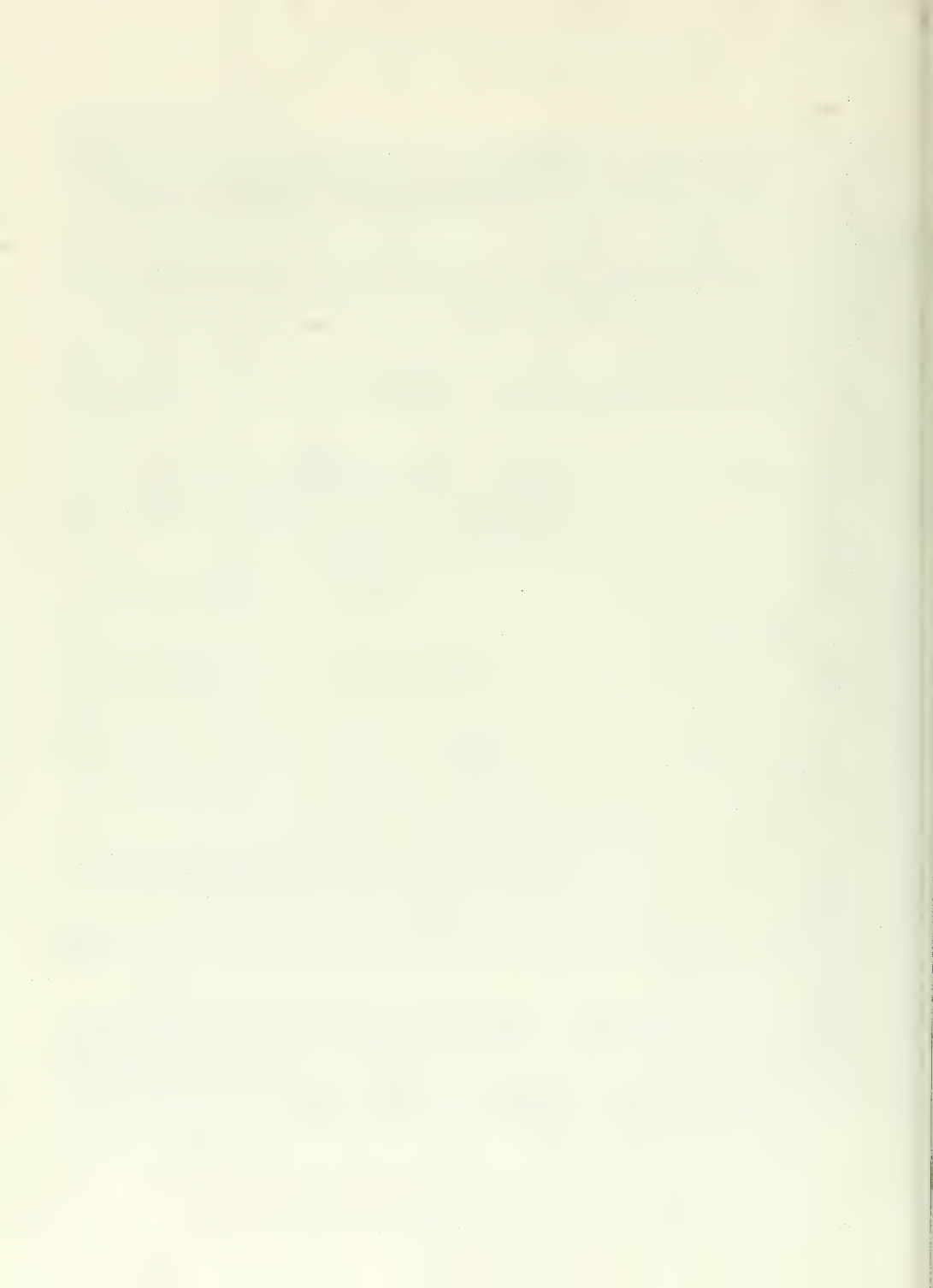
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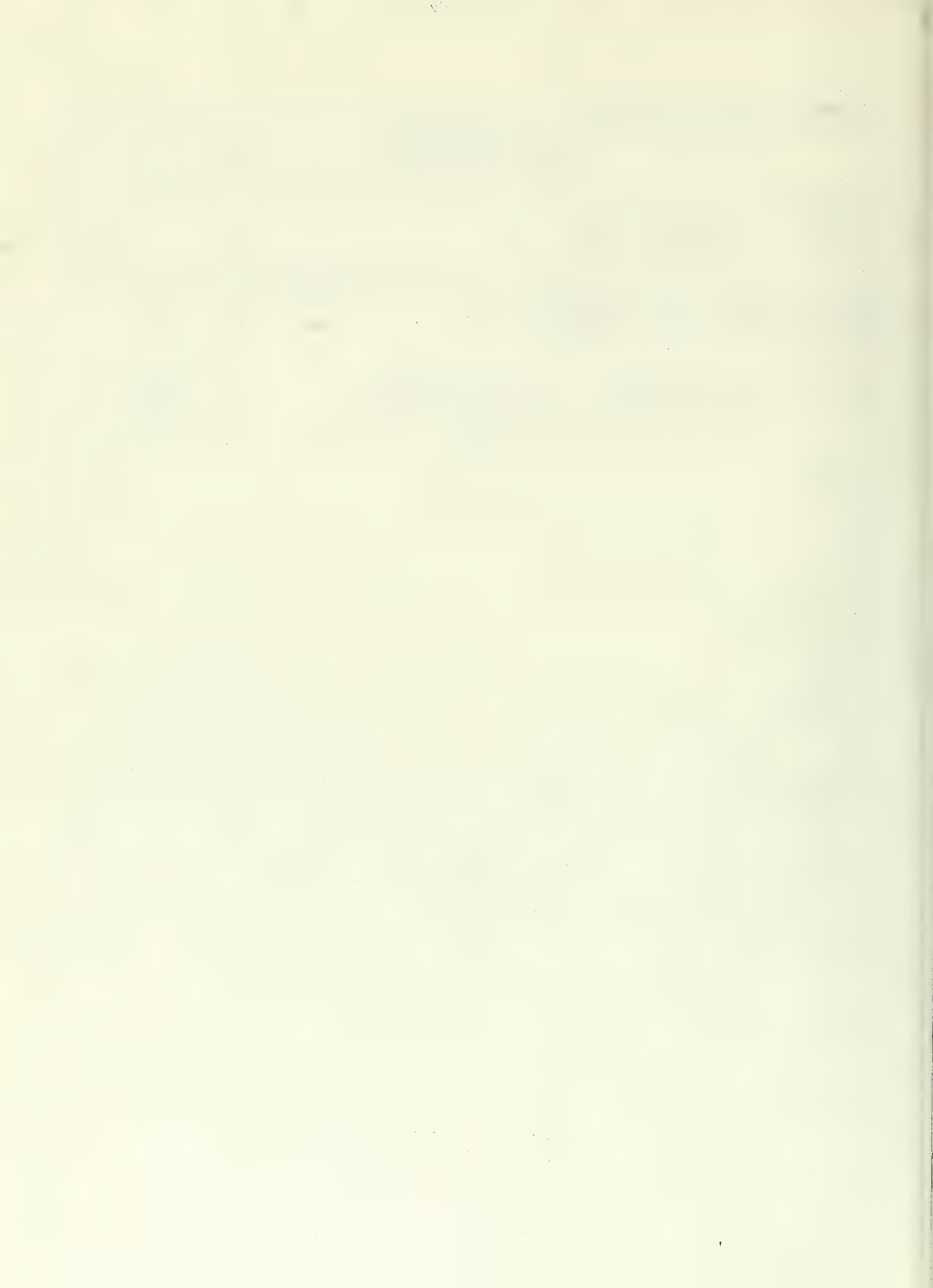
Mendelson, W.B.: An assessment of federal and private funding of sleep research. Sleep (in press).

Gillin, J.C., Mendelson, W.B., and Sitaram, N.: Acetylcholine, sleep, and depression. Hum. Neurobiol. (in press).

Sitaram, N., Kaye, W.H., Nurnberger, J.I., Ebert, M., Gershon, E.S., and Gillin, J.C.: Cholinergic REM sleep induction -- A trait marker of affective illness. In: I. Hanin and E. Usdin (Eds.)



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|--|---|--|-----------------|-------------------|-----------------|-----|------|--------|------------------------|-----------------|----|------|--|---------------------|---------------|----|------|--|-------------------------|------------------------|----|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00098-08 BP | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Study of Children and Grandchildren of Patients with Depressive Illness | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
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| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <u>Biological Psychiatry Branch</u> | | | | | | | | | | | | | | | | | | | | | | |
| SECTION <u>Unit on Childhood Mental Illness</u> | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <u>NIMH, NIH, Bethesda, Maryland 20205</u> | | | | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been discontinued. | | | | | | | | | | | | | | | | | | | | | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00100-07 BP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Biobehavioral Aspects in Childhood and Adolescent Mental Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS, DCBR, NIMH; Communicative Disorders Program, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Office of the Chief; Unit on Childhood Mental Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) An inpatient program with selected overnight stays, for <u>childhood and adolescent neuropsychiatric disorders</u> is on- going. The condition currently under study is that of hyperactive children (HAC). Pharmacological compounds under study in these disorders include <u>methyl-</u> <u>phenidate</u> , <u>amphetamine</u> , <u>piribedil</u> , <u>L-dopa</u> , <u>tryptophan</u> , <u>Mianserin</u> , and <u>clorgy-</u> <u>line</u> . Piribedil is safe but clinically ineffective in HAC. L-dopa is minimally clinically effective in HAC. <u>Pharmacokinetic</u> studies with clinical responses are included. Amphetamine half-life in children is about one-third that of adults. Behavior and motor activity responses to d-amphetamine occur during the absorp- tion phase as determined by serial plasma amphetamine following a single dose. <u>Central neurotransmitters</u> and their metabolites are being studied in plasma and urine. Urinary <u>3-methoxy-4-hydroxyphenylglycol</u> shows a time-related decrease during treatment with d-amphetamine; homovanillic acid is unchanged. In general, norepinephrine metabolites in urine are decreased after two weeks of d-amphe- tamine vs. placebo and dopamine metabolites are not. Tyramine and its metabo- lites are also decreased following d-amphetamine, whereas phenylethylamine is greatly increased following d-amphetamine. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: The purposes of this program are broad. An objective is to gain new knowledge of the central nervous system (CNS) of children and adolescents with special reference to maturational changes and neuropsychiatric disorders. Compared to the neurobiology known in adult neuropsychiatry, much less is known regarding the neuropsychiatric disorders of children. A particular focus of these studies has been the relationship between neurotransmitter change in hyperactive children (HAC) following compounds that have major actions on central neurotransmitter metabolism. The study of d-amphetamine (d-AMPH), a compound with clear and reliable effects on HAC, has been of particular interest, particularly its pharmacokinetics, its effects on catecholamine and indoleamine metabolism, its behavioral effects, and the interrelationships of these effects.

There have been a number of hypotheses relating catecholamine metabolism and hyperactivity in children. The possibility of an overly active catecholaminergic system was first advanced. Later, a functional deficiency in catecholamines in HAC was proposed with the greater focus on the possibility of a functional dopamine (DA) rather than norepinephrine (NE) deficiency, a hypothesis based on the following: 1) possible decreased functioning of the reward-system median forebrain bundle; 2) behavior in children with Von Economo's encephalitis resulting in a DA deficiency; 3) action on AMPH and its cyclized derivative, methylphenidate, both of which release NE and DA among other pharmacological actions; and 4) specificity of biochemical pharmacological interactions such as the proposed differences in the mode of action of d- and l-AMPH. This latter distinction was taken to indicate that DA deficiency might be of more importance than NE deficiency, based on the hypothesis that d-AMPH is a more potent re-uptake blocker of NE than l-AMPH. Whereas d-AMPH also was thought to be more potent in releasing NE at the synapse, l-AMPH appeared to affect DA and NE equally. However, a number of other studies question whether the differential effects of d- and l-AMPH can be used to distinguish NE and DA metabolism. Other biochemical alterations, particularly involving serotonin (5-HT) have been proposed.

Methods Employed: An inpatient and day patient program for children and adolescents, involving selected overnight stays, is ongoing on an Inpatient Nursing Unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties have been admitted in order to study a carefully defined population of HAC. Children and adolescents with other conditions have also been studied. Specific exclusion and inclusion criteria are employed.

All children are thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Children also receive a psycholinguistic examination in collaboration with NINCDS. Neurological examinations are scored carefully according to a rating scale (PANESS). Several clinical and behavioral rating instruments have been utilized.

Pharmacological compounds, both standard and those previously unused in children, are being studied. Serial plasma pharmacokinetic data are being generated for d-AMPH. These data are studied in conjunction with motor activity, behavior, cognition, speech, temperature, and cardiovascular response. Piribedil, a

specific DA agonist, and L-dopa have been given to HAC. Mianserin, a NE agonist; tryptophan (TP), a precursor of 5HT; and clorgyline, a monoamine oxidase (MAO)A inhibitor have all been administered to HAC as clinical trials.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. At any time the instrument can be read into a computer for a print-out. Behavioral changes in HAC are measured via Conners' Teachers' Rating Scale (CTRS). Cognition is measured by a continuous performance task (CPT) in which errors of omission and commission can be scored in terms of differing time intervals. Time intervals can also be increased or decreased in relationship to accuracy of response.

D-AMPH is measured by a radioimmunoassay (RIA) and gas chromatograph mass spectrometry (GC-MS). Biochemical studies include 24-hour urine collection to study NE, 3-methoxy-4-hydroxyphenylglycol (MHGP), vanillylmandelic acid (VMA), and normetanephrine (NMN); DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT); tyramine (TRM) and parahydroxyphenylacetic acid (PHPA); and phenylethylamine (PEA) and phenylacetic acid (PAA). Plasma pharmacokinetics of pharmacological compounds are being ascertained. Plasma NE, MHGP, NMN, and VMA changes as they relate to plasma d-AMPH levels have also been studied. Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Average evoked response (AER) studies are being conducted as they relate to HAC in drug-free and treated conditions. Psycholinguistic changes are also studied in relation to d-AMPH, piribedil, and TP effects. Paired associate learning has also been assessed in different drug conditions. Chronic effects of d-AMPH (2 weeks) are being studied with regard to pharmacokinetics and clinical response, particularly in terms of an evidence for tolerance or supersensitivity and effect on catecholamine metabolism, as manifested by changes in plasma NE and dopamine-beta-hydroxylase (DBH) and platelet 5HT and MAO. The effects of TP and valine and d-AMPH and placebo are being measured with regard to behavior, motor activity, and plasma amino acids and indoleamines.

Major Findings: Significant correlations exist between community teachers' and NIH teachers' CTRS ratings of behavior.

Serial plasma pharmacokinetic data indicate that children reach a peak plasma level of d-AMPH within 3-4 hours (h) of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 ± 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data is highly replicable. Clinical responses have also been studied after use of sustained release capsules. This preparation produces a slower rate of absorption and a more plateau-like, longer lasting peak level, but does not give a prolonged clinical response. Socially appropriate behavioral change and motor activity change is maximal at 1-3 h after administration of a single dose (0.5 mg/kg) of d-AMPH which corresponds to its absorption phase. This data indicates that the clinical changes may be related to release of catecholamines and the subsequent depletion of their stores, replacement by a "false" neurotransmitter metabolite of AMPH, or to alteration in receptor sensitivity. Higher

single doses (1.0 mg/kg) effect similar clinical responses, but less so. Piribedil is safe, but clinically ineffective for HAC. Preliminary results indicate that neither TP nor valine (a neutral amino acid which competes with TP and inhibits its crossing the blood-brain barrier) result in behavioral response after a single dose; on the other hand, d-AMPH after a single dose appears to have no effect on serial plasma amino acids, 5HT, or 5-hydroxyindoleacetic acid (5HTAA) over a six-hour period. This preliminary finding could be quite important in that d-AMPH has been shown to have clear effects in central 5HT in animals.

Studies of urinary metabolites indicate that day and night excretion of MHPG and HVA are not significantly different; however, d-AMPH after eight and fourteen days is associated with significantly lower MHPG levels and the data indicates that behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged. These biochemical findings have been replicated in a subsequent HAC group, as well as extended to other metabolites of both NE and DA. TRM and PHPA excretion are significantly decreased following two weeks of d-AMPH. PEA excretion is markedly elevated following two weeks of treatment with d-AMPH.

HAC are not significantly different from normal controls with regard to plasma NE and DBH but do not have significantly more neurological soft signs by PANESS examination. Plasma NE is significantly correlated with anxiety as well as being significantly elevated at 2-3 h following a single oral dose of d-AMPH. The change in plasma NE correlates with the change in plasma d-AMPH. The elevated plasma NE is associated with increases in blood pressure and pulse and is dose-related. Of related interest, baseline plasma NE, measured prior to an early a.m. dose of d-AMPH, does not change after two weeks of d-AMPH versus two weeks of placebo.

Preliminary results indicate a decreased platelet 5HT and increased platelet MAO in HAC vs. normals; however platelet MAO also correlates positively and significantly with age.

Studies evaluating CPT are now underway; d-AMPH is effective and piribedil and L-dopa are minimally so. AER studies are preliminary. HAC with higher levels of soft signs have more abnormal EEG's, more minor physical anomalies, lower full scale IQ's (WISC-R), and a greater number of errors on the Bender. Data from psycholinguistic evaluations are being prepared for publication. Of most clinical interest is the finding that d-AMPH appears to have a therapeutic effect on certain speech difficulties.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly HAC, have been considerably studied in the last few years, there are many diagnostic, psychopharmacological, and psychobiological questions to be answered. Many studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards HAC, obsessive-compulsive children, enuretics, Gilles de la Tourette's syndrome, anorexia nervosa, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and AMPH give positive responses in 80% of well diagnosed HAC, the pharmacokinetics and metabolism of these drugs has not been closely studied in these children. One avenue to ascertaining possible neuro-

pathology in these conditions is to understand more clearly the mechanisms of action of those pharmacological compounds which effectively alter the clinical conditions under study. The relationship between such basic pharmacological knowledge and clinical effects has been under-studied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in understanding the biological contributions to childhood neuropsychiatry.

Proposed Course of Project: There is a considerable body of data yet to be analyzed and reported, and this line of investigation is likely to continue over the next two years.

Publications:

Langer, D.H., Rapoport, J.L., Brown, G.L., Ebert, M.H., and Bunney, W.E., Jr.: Questioning a dopaminergic hypothesis. Am. J. Psychiatry 138(4): 537, 1981.

Mikkelsen, E.J. Lake, C.R., Brown, G.L., Ziegler, M.G., and Ebert, M.H.: The hyperactive child syndrome: Peripheral sympathetic nervous system function and the effect of d-amphetamine. Psychiatry Res. 4(2): 157-169, 1981.

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Langer, D.H., Fletcher, J.C., Brown, G.L., Nee, L.E., and Smith, M.A.: Ethical considerations in psychological research in children. In Greenhill, L. and Shopsin, B. (Eds.): The Psychobiology of Childhood: Profiles of Current Issues. New York, Spectrum Publications, in press.

Brown, G.L., and Ebert, M.H.: Catecholamine metabolism and hyperactive children. In Lake, C.R., and Ziegler, M.G. (Eds): Norepinephrine: Clinical Aspects. Baltimore, Williams and Wilkins, in press.

Ludlow, C., Cudshy, E., Bassich, C., and Brown, G.L.: The auditory processing skills of hyperactive, language impaired and reading disabled boys. In Katz, J., and Laskey, E.Z. (Eds.): Central Auditory Processing Disorders: Problems of Speech, Language, and Learning. Baltimore, University Park Press, in press.

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| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Treatment of Obsessional Children and Adolescents with Chlorimipramine | | | | | | | | | | | | | | | | | | | | | | |
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| | Dennis L. Murphy, M.D. | Chief | CN | NIMH | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Clinical Neuropharmacology Branch, DCBR, NIMH | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <u>Biological Psychiatry Branch</u> | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Unit on Childhood Mental Illness | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | | | | | | |
| .25 | .25 | .25 | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Obsessional disorder of childhood is a rare disorder about which little is known. In this protocol we have collected basic data on family history of mental illness, sleep measures, CAT Scans and neuropsychological testing on seventeen children and adolescents with obsessional disorder, and age/sex/handedness matched controls. Major findings to date are a higher Ventricular Brain Ratio (VBR), and decreased visuo-spatial ability in patients compared with controls. Clinically, these children did have mild pre-morbid temperament, but had few obsessive-compulsive traits before developing the disorder; state and trait appear discontinuous. A drug trial of <u>chlorimipramine</u> or placebo has been carried out to evaluate the effect of the <u>antidepressant</u> and the specificity of chlorimipramine for this disorder. | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: (1) To examine clinical, family, physiological, neuroradiological and neuropsychological measures in childhood Obsessive-Compulsive Disorder; (2) To evaluate drug treatment of this condition. Clinical response to chlorimipramine will be related to platelet serotonin at baseline, plasma drug concentration and baseline severity of obsessive-compulsive and depressive symptoms.

Methods Employed: Patients are sought on a national level because of the rarity of the disorder. Inclusion criteria are Obsessive-Compulsive Disorder as a primary disturbance. Children must have IQ of 85 or above, and be free from known neurological disturbance or psychosis.

A modification of the Leyton Obsessional Inventory is used to monitor drug effects on Obsessive-Compulsive symptomatology throughout the 12-week clinical trial. Weekly ratings are made by two physicians and ward nurses on the CPRS.

In addition to routine psychological testing, children were given the Neuro-sensory Center Examination For Apasia (NCEAA), Money's Road Map Test of Directional Sense and the Stylus Maze Test (Milner).

Plasma levels of drug as well as platelet serotonin are assayed for each drug phase.

Major Findings: Seventeen children have entered the protocol: fourteen males aged 13-17, and three females aged 13 (1) and 15 (2). This is a strikingly ill group of children for whom state is discontinuous with trait. Patients improved gradually throughout their hospital course and as a group did not have a different course during chlorimipramine treatment. Depression ratings changed in parallel with obsessive symptoms. One patient showed dramatic improvement on chlorimipramine and was being maintained on the drug for six months. The others have showed equivocal responses.

A problem in interpreting drug effects is the tendency for there to be gradual improvement over the course of the study for almost all of the patients. This group trend obscures individual drug/placebo differences and may mask true drug effects. Intensive studies of "responders" are planned.

Preliminary analysis of family pedigree data is somewhat at variance with other reports of Obsessive-Compulsive Disorders. Only one patient reports obsessive-phobic symptomatology in any family members. The other families have no obsessional illness among first degree relatives. There were a surprising number of siblings who exhibit learning and behavior problems. Six of the 19 siblings age 18 and under have such symptoms. Sleep recordings on the group showed a decrease in REM latency at baseline, which increased over time with clinical improvement. These findings indicate a possible biologic link between depression and Obsessive-Compulsive Disorder in childhood.

Obsessives appear to have some rather specific neurolinguistic and neuropsychological deficits. While memory and attention did not differ significantly between the groups, obsessives performed significantly more poorly on a dichotic listening task using syllables ($p < .01$), and did more poorly on a tactile naming task ($p < .01$). As a group, the patients also performed more poorly on the Road Map Test ($p < .006$) and the Stylus Maze Test ($p < .0002$).

Preliminary data from measurements of ventricular size on the CT Scans shows that obsessives tend to have a higher Ventricular Brain Ratio (VBR) than age- and sex-matched controls ($p < .05$).

Platelet MAO and 5HT from the patients and a group of matched adolescent controls were not significantly different.

Significance to Mental Health Research: Obsessive-Compulsive Disorder is a rare but extremely disabling condition. About one third of adults with the disorder had their onset during childhood or adolescence. Children with the condition are often very ill and do not respond well to psychodynamic treatment. Relative to other rare but disabling conditions, such as infantile autism, there has been virtually no research in this area of childhood mental illness.

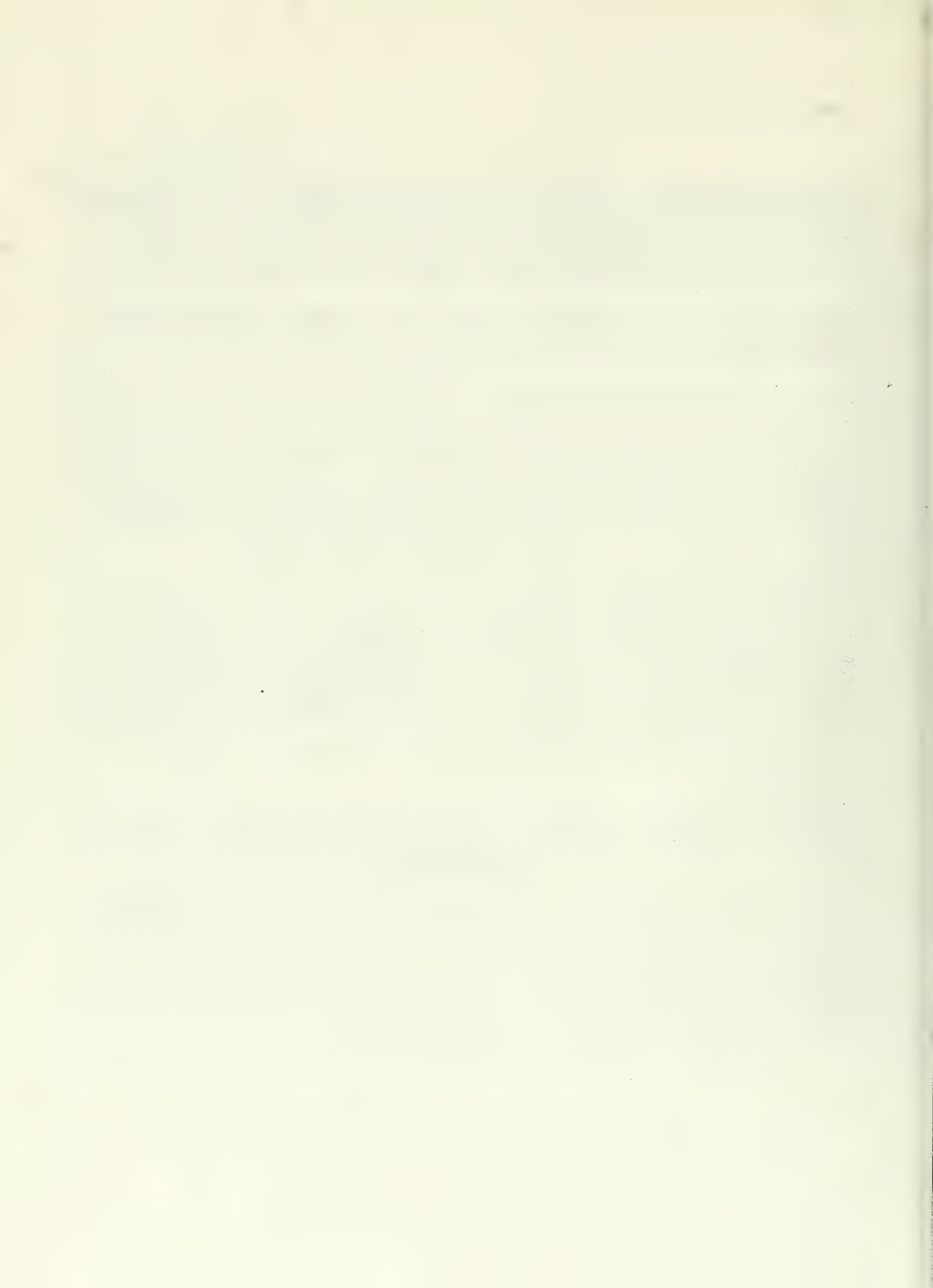
Proposed Course of Project: A total of 20 patients will be studied to complete the chlorimipramine trial. Baseline clinical measures, plasma tricyclic level, and platelet serotonin will be examined in relation to clinical response to the active drugs. Neuropsychological and linguistic testing will be compared for patients and controls. A long-term follow-up is planned for all subjects, with plans to have them undergo PET Scans when over 18. Drug trials will be continued with other pharmacologic agents such as zimelidine, amphetamine and Parnate, all reported useful with adult patients.

Publications:

Rapoport, J., Elkins, R., Langer, D., Sceery, W., Buchsbaum, M., Gillin, J. C., Murphy, D. L., Zahn, T., Lake, R., Ludlow, C., and Mendelson, W.: Childhood Obsessive-Compulsive Disorder. Am. J. Psychiat. 138: 1545-1554, 1981.

Berg, C., Zahn, T., Behar, D., Rapoport, J.: Childhood Obsessive-Compulsive Disorder: An Anxiety Disorder. In Gittelman, R. (Ed.): Anxiety in Children. New York, Guilford Press. In press.

Rapoport, J.: Childhood Obsessive-Compulsive Disorder. In: Shuffer, D., Ehrhardt, A. Greenhill, L. (Eds.): Diagnosis and Treatment in Pediatric Psychiatry. New York, McMillan. In press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00161-04 BP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Cognitive Effects of Dietary Substances in Normal and Hyperactive Children | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Judith L. Rapoport, M.D. Other: David Behar, M.D. Alan Neims, Ph.D. Marvin Cornblath, M.D. | Chief, Unit on Childhood Mental Illness Clinical Associate Professor of Pharmacology and Pediatrics, U. of Florida, Gainesville, Florida Chief, Pediatric Medicine Branch | BP NIMH BP NIMH NICHD |
| COOPERATING UNITS (if any) Laboratory of Psychology and Psychopathology, DCBR, NIMH Section on Experimental Therapeutics, LCS, DCBR, NIMH; U. of Fla., Gainesville PMB, NICHD | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Unit on Childhood Mental Illness | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: .40 | PROFESSIONAL: .30 | OTHER: .10 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Both <u>caffeine</u> and <u>sugar</u> are common <u>dietary</u> substances that have been implicated in behavioral and cognitive deficits in a variety of adult populations. The effects of these substances on normal and behaviorally deviant children are being studied in an ongoing series of studies. Caffeine in relatively high doses (10mg/kg) may increase nervousness and insomnia as well as worsen classroom behavior. However, preliminary evidence indicates that children who habitually self select high caffeine diets are not adversely affected and may even benefit from caffeine. Current studies of behavioral response to <u>glucose</u> , <u>sucrose</u> and <u>artificial sweetener placebo</u> do not show any significant behavioral effects on grade school children thought to be sensitive to sugar. | | |

Project Description:

Objectives: To evaluate the effects of caffeine (4 and 10 mg/kg) in single doses and subacute effects (10 mg/kg/day for 2 weeks) on activity, memory and vigilance of normal children selected for habitual high and low caffeine consumption. To evaluate effects of glucose and sucrose (1.75 gm/kg) on activity, memory and behavior of normal hyperactive children thought to be intolerant to sugars by their parents.

Methods Employed: Children are paid as normal volunteers for participating in these studies. Children in the caffeine study were free from psychiatric disorder or cognitive disability. Children in the sugar study were diagnosed as having Attention Deficit Disorder, and considered by parents and/or pediatricians to have carbohydrate sensitive behavior disorders.

Double-blind single dose and subacute trials of caffeine were carried out, using cognitive test response as the dependent measures.

Double-blind studies of single doses of caffeine (3 mg/kg and 10 mg/kg) were conducted with 19 grade school boys. A second study was carried out of the subacute effects of caffeine (10 mg/kg day). In this latter study, subjects had daily caffeine or placebo for two-week periods in a double-blind crossover design. For the sugar studies, we devised behavioral glucose, sucrose and saccharin tolerance tests. Children were followed with serial increases of activity, attention, behavior ratings, and blood glucose, insulin and cortisol.

Major Findings:

Acute caffeine study: Single doses of caffeine (10 mg/kg) had several significant effects on the normal children's cognitive and behavioral responses. In contrast to placebo or the lower dose (3 mg/kg), high doses of caffeine increased motor restlessness while improving vigilance, as measured by reaction time and continuous performance test.

The first subacute caffeine study with a separate sample of 19 boys has been completed. During the caffeine period, parents and teachers rate the children as more restless, more nervous and jittery. These findings have implications for the biological basis of anxiety. However, children who were habitual low caffeine consumers were most likely to suffer adverse effects, while habitual high consumers were somewhat calmed on caffeine. This study is being replicated on a large scale, with a two week off caffeine baseline to avoid withdrawal effects, and with epidemiological sampling.

Sugar Study: The data are still being analyzed. Preliminary analysis shows no significant behavioral effects of sugar for the group as a whole.

Significance to Mental Health Research: There is little known about the influence of diet on normal children's behavior. Caffeine is widespread in our daily diet and the influence of dietary level on both normal and pathological samples has considerable public health significance. Theoretically, the effects of caffeine resemble mild clinical anxiety, and caffeine may provide a pharmacological model for anxiety in childhood.

Proposed Course of Project: A large double-blind outpatient study is in progress with 30 high caffeine consuming children selected from a survey of 800 grade school students, and 30 age/sex/classroom matched, low caffeine consuming controls. These groups will be compared in a double-blind crossover study of caffeine (10mg/kg/day) for two weeks or placebo. Classroom and home behavior as well as mood will be examined across the two groups, and between caffeine and placebo periods.

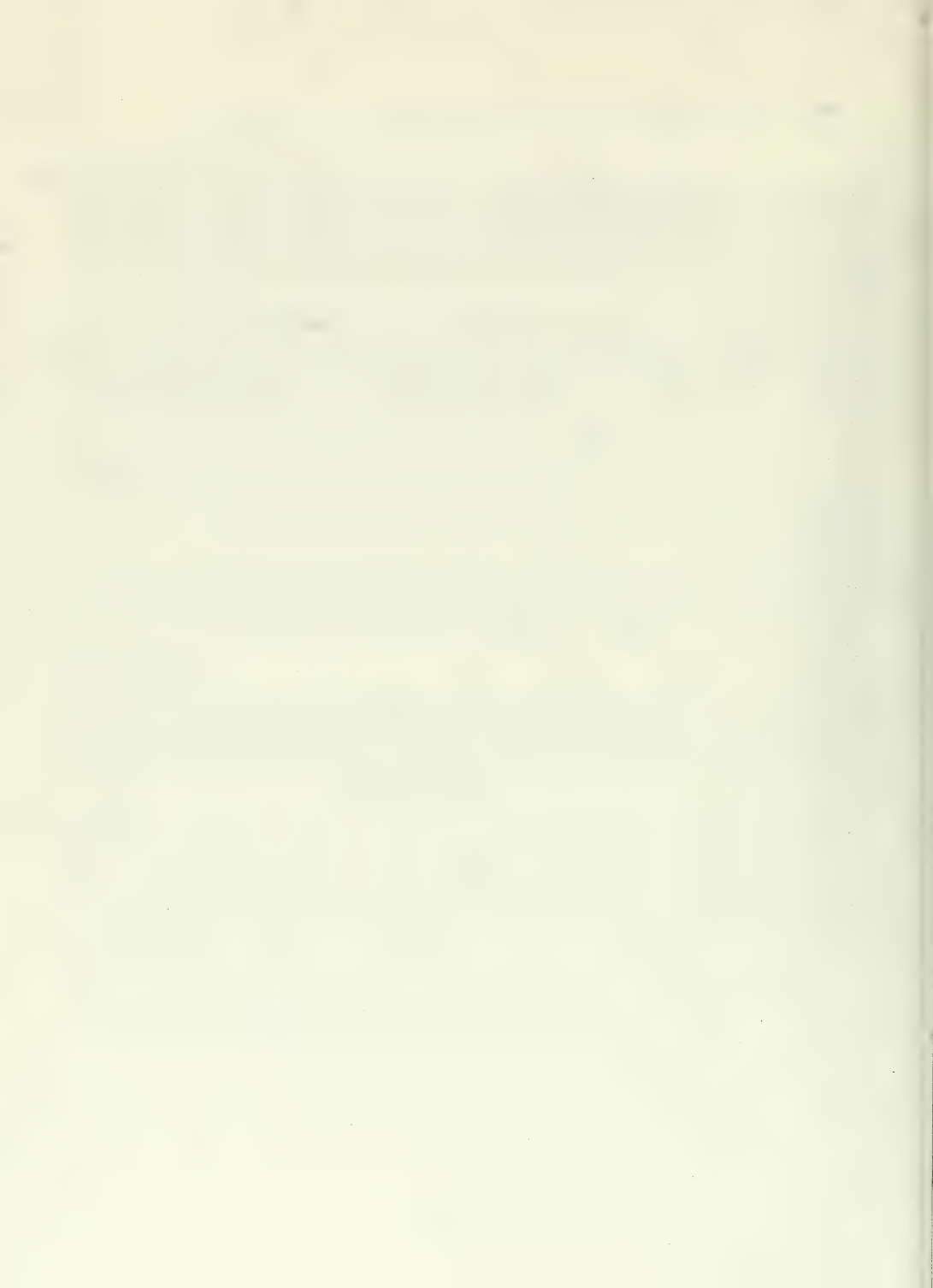
The few sugar sensitive subjects are being retested with repeated sugar and placebo challenges. If these subjects show "positive" response on repeat testing, trial dietary management will be conducted on an individual basis.

Publications:

Rapoport, J., Elkins, R., Zahn, T., Buchsbaum, M., Weingartner, H., and Kopin, I.: Acute effects of caffeine on normal prepubertal boys. In Klein, D.F. and Robkin, J. (Eds.): Anxiety. New Research and Changing Concepts. New York, Raven Press, 1981, pp. 335-366.

Rapoport, J., Elkins, R., Neims, A., Zahn, T., and Berg, C. Behavioral and autonomic effects of caffeine in normal boys. *Devel. Pharmacol.* 3: 74-82, 1981.

Rapoport, J., Jensvold, M., Elkins, R., Buchsbaum, M., Weingartner, H., Ludlow, C., Zahn, T., Berg, C., and Neims, A. Behavioral and cognitive effects of caffeine in boys and adult males. *J. Nerv. Ment. Dis.* 169: 726-732, 1981.



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|--|---|--|----------|--------------------------|-----------------------------|------|--|--|----------------|--|--------|---------------|--------------------|---------|--|-------------------|---------------------|---------|--|--|-----------------------|--|--|-----------------------------|--------------------|---------|--|---------------------|-------------------|----------|--|--|---------------------------|--|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00162-03 BP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Treatment of Hyperactive Children with Desmethylinipramine | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Judith L. Rapoport, M.D.</td> <td style="width: 33%;">Chief, Unit on Childhood BP</td> <td style="width: 33%;">NIMH</td> </tr> <tr> <td></td> <td></td> <td style="padding-left: 20px;">Mental Illness</td> <td></td> </tr> <tr> <td>Other:</td> <td>Alan Zametkin</td> <td>Clinical Associate</td> <td>BP NIMH</td> </tr> <tr> <td></td> <td>Steven Paul, M.D.</td> <td>Chief, Unit on Pre-</td> <td>CB NIMH</td> </tr> <tr> <td></td> <td></td> <td style="padding-left: 20px;">clinical Pharmacology</td> <td></td> </tr> <tr> <td></td> <td>William Potter, M.D., Ph.D.</td> <td>Assistant to Chief</td> <td>CP NIMH</td> </tr> <tr> <td></td> <td>Michael Ebert, M.D.</td> <td>Chief, Section on</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td></td> <td style="padding-left: 20px;">Experimental Therapeutics</td> <td></td> </tr> </table> | | | PI: | Judith L. Rapoport, M.D. | Chief, Unit on Childhood BP | NIMH | | | Mental Illness | | Other: | Alan Zametkin | Clinical Associate | BP NIMH | | Steven Paul, M.D. | Chief, Unit on Pre- | CB NIMH | | | clinical Pharmacology | | | William Potter, M.D., Ph.D. | Assistant to Chief | CP NIMH | | Michael Ebert, M.D. | Chief, Section on | LCS NIMH | | | Experimental Therapeutics | |
| PI: | Judith L. Rapoport, M.D. | Chief, Unit on Childhood BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | Mental Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Other: | Alan Zametkin | Clinical Associate | BP NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Steven Paul, M.D. | Chief, Unit on Pre- | CB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | clinical Pharmacology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | William Potter, M.D., Ph.D. | Assistant to Chief | CP NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Michael Ebert, M.D. | Chief, Section on | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | Experimental Therapeutics | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Laboratory of Sleep Physiology, DCBR, IRP, NIMH Clinical Psychobiology Branch, DCBR, IRP, NIMH Laboratory of Clinical Science, DCBR, IRP, NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Unit on Childhood Mental Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| .65 | .40 | .25 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) A trial of <u>desmethylinipramine</u> is planned to compare the acute and chronic efficacy of this agent in <u>hyperactive children</u> and to relate clinical effects to measures of <u>norepinephrine metabolism</u> . | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: As tricyclic antidepressants have been shown to have short-term beneficial effects for hyperactive children, this study is designed to examine these effects more closely. Desmethylinipramine (DMI) acts primarily by blocking reuptake of norepinephrine (NE). By monitoring plasma NE, urinary MHPG and examining clinical effects, changes in NE metabolism and antihyperactive effects could be examined to see if they are related.

Methods Employed: DMI or placebo are being tried in a 3-week double-blind study; a final sample of 24 children is planned.

Major Findings: DMI has a weak antihyperactive effect. The sample is still too small to permit further data analysis.

Significance for Mental Health Research: Hyperactivity is a major issue in child psychiatry. Understanding its mechanism may lead to more effective treatment and prevention.

Projected Course of Project: The protocol will continue using DMI and placebo in a double-blind non-crossover design. Clinical efficacy of DMI will be examined in relation to chronic (1 month) drug, and chemical effects will be compared and related to clinical effects. Approximately eight more children will be studied.

Publication:

Rapoport, J., Langer, D., and Ebert, M.: Pilot trial of mianserin treatment of hyperactive boys. In Greenhill, L. (Ed.): Biological Aspects of Child Psychiatry. New York, Spectrum Publishers, 1982, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00163-03 BP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) <u>Naturalistic Study of Activity Levels of Hyperactive Children</u> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| P.I. Linda Porrino, Ph.D. Other: Judith Rapoport, M.D. Thomas Wehr, M.D. David Behar, M.D. | Guest Worker Unit on Childhood Mental Illness Chief, Unit on Childhood Mental Illness Chief, Clinical Research Unit Clinical Associate | BP NIMH BP NIMH CP NIMH BP NIMH |
| COOPERATING UNITS (if any) Clinical Psychobiology Branch, DCBR, NIMH | | |
| LAB/BRANCH <u>Biological Psychiatry Branch</u> SECTION <u>Unit on Childhood Mental Illness</u> | | |
| INSTITUTE AND LOCATION <u>NIMH, NIH, Bethesda, Maryland 20205</u> | | |
| TOTAL MANYEARS: .65 | PROFESSIONAL: .40 | OTHER: .25 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Because of the new technology for measuring <u>24 hour activity</u> using the NIH actometer, a study has been conducted to examine activity levels of <u>hyperactive</u> children and matched controls at baseline. Following this, activity of the hyperactive group was compared during <u>amphetamine</u> and placebo treatment periods. This was the first study to examine drug effects on motor activity outside of a laboratory setting. Drug effects were examined in relation to measures of structure of classroom and home environment. | | |

Project Description:

Objectives: Hyperactive boys and matched controls who are in the same school, grade and classes were followed for a baseline week to compare 24 hour activity patterns for the groups. Following this, hyperactive boys were monitored continuously for four weeks; the effects of amphetamine (25 mg) or placebo were compared in a double-blind ABAB design.

Methods Employed: Activity levels during school, free play and home activities were compared and related to measures of attention (Continuous Performance Test), and ratings of school and home structure. Children were monitored on belts even when they slept. Weekly appointments were kept at which time parent and teacher behavior ratings, side effects, hourly diaries of weekly activities and attentional measures were obtained.

Major Findings: A total of 12 patient-control pairs were studied. Findings indicate that hyperactive children are significantly more restless than controls even during sleep. Hyperactives are more active during a variety of activities - the group differences were most striking during school but were also significant after school and on weekends. Motor activity differentiated the groups as well as did attentional tasks. Drug effects appear biphasic, decreasing activity during the day with some increase in activity in the evening. This "rebound" effect may represent altered receptor sensitivity and has not been reported elsewhere in clinical pharmacology.

Significance to Mental Health Research: Only a naturalistic study can relate laboratory findings to clinically relevant situations. The nature of "hyperactivity" is poorly understood. Since hyperactive children are truly more restless than their peers, it is important to know for what situations this is true. Furthermore, this study will provide data on behavioral "rebound" in the evenings following drug. If this is a major finding, then altered dose schedule would be indicated.

Proposed Course of Project: Further studies with different drug dose and schedules are planned.

Publications: Several submitted.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00164-03 BP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) A Study of Infants of Parents with Bipolar Affective Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 50%;">Leon Cytryn, M.D.</td> <td style="width: 20%;">Medical Officer</td> <td style="width: 10%;">BP</td> <td style="width: 10%;">NIMH</td> </tr> <tr> <td>Other:</td> <td>Yolande Davenport, M.S.W.</td> <td>Social Worker</td> <td>LDP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Martine Lamour, M.D.</td> <td>Guest Worker</td> <td>BP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Donald H. McKnew, M.D.</td> <td>Medical Officer</td> <td>BP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Marian Yarrow, Ph.D.</td> <td>Research Psychol.</td> <td>LDP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Carolyn Waxler, Ph.D.</td> <td>Research Psychol.</td> <td>LDP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Ann Barnett, M.D.</td> <td colspan="3">Children's Hospital Washington, D.C.</td> </tr> <tr> <td></td> <td>Ira Weiss, Ph.D.</td> <td colspan="3">Children's Hospital Washington, D.C.</td> </tr> <tr> <td></td> <td>Robert Harmon, M.D.</td> <td colspan="3">Univ. of Colorado, Denver, Col</td> </tr> <tr> <td></td> <td>Theodore J. Gaensbauer, M.D.</td> <td colspan="3">Univ. of Colorado, Denver, Col.</td> </tr> <tr> <td></td> <td>Frederick Goodwin, M.D.</td> <td>Scientific Director</td> <td>IRP</td> <td>NIMH</td> </tr> </table> | | | PI: | Leon Cytryn, M.D. | Medical Officer | BP | NIMH | Other: | Yolande Davenport, M.S.W. | Social Worker | LDP | NIMH | | Martine Lamour, M.D. | Guest Worker | BP | NIMH | | Donald H. McKnew, M.D. | Medical Officer | BP | NIMH | | Marian Yarrow, Ph.D. | Research Psychol. | LDP | NIMH | | Carolyn Waxler, Ph.D. | Research Psychol. | LDP | NIMH | | Ann Barnett, M.D. | Children's Hospital Washington, D.C. | | | | Ira Weiss, Ph.D. | Children's Hospital Washington, D.C. | | | | Robert Harmon, M.D. | Univ. of Colorado, Denver, Col | | | | Theodore J. Gaensbauer, M.D. | Univ. of Colorado, Denver, Col. | | | | Frederick Goodwin, M.D. | Scientific Director | IRP | NIMH |
| PI: | Leon Cytryn, M.D. | Medical Officer | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Other: | Yolande Davenport, M.S.W. | Social Worker | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Martine Lamour, M.D. | Guest Worker | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Donald H. McKnew, M.D. | Medical Officer | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Marian Yarrow, Ph.D. | Research Psychol. | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Carolyn Waxler, Ph.D. | Research Psychol. | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Ann Barnett, M.D. | Children's Hospital Washington, D.C. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Ira Weiss, Ph.D. | Children's Hospital Washington, D.C. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Robert Harmon, M.D. | Univ. of Colorado, Denver, Col | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Theodore J. Gaensbauer, M.D. | Univ. of Colorado, Denver, Col. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Frederick Goodwin, M.D. | Scientific Director | IRP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) LDP, NIMH; University of Colorado, Denver, Colorado; Children's Hospital, Washington, D.C.; Clinical Psychobiology Branch, DCBR, NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Unit on Childhood Mental Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 1.4 | OTHER: 1.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been discontinued. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |



Project Description:

Objectives: There is evidence for a genetic factor particularly among male alcoholics. This pilot project compared blood and breath acetaldehyde for "high risk" and control children.

Methods Employed: Extensive recruitment through area alcohol treatment programs has produced a sample of approximately 11 high risk children and 11 age-matched controls. Clinical screening and skin biopsy were completed. A challenge of alcohol (0.5 m./kg) with clinical and biological measures was completed.

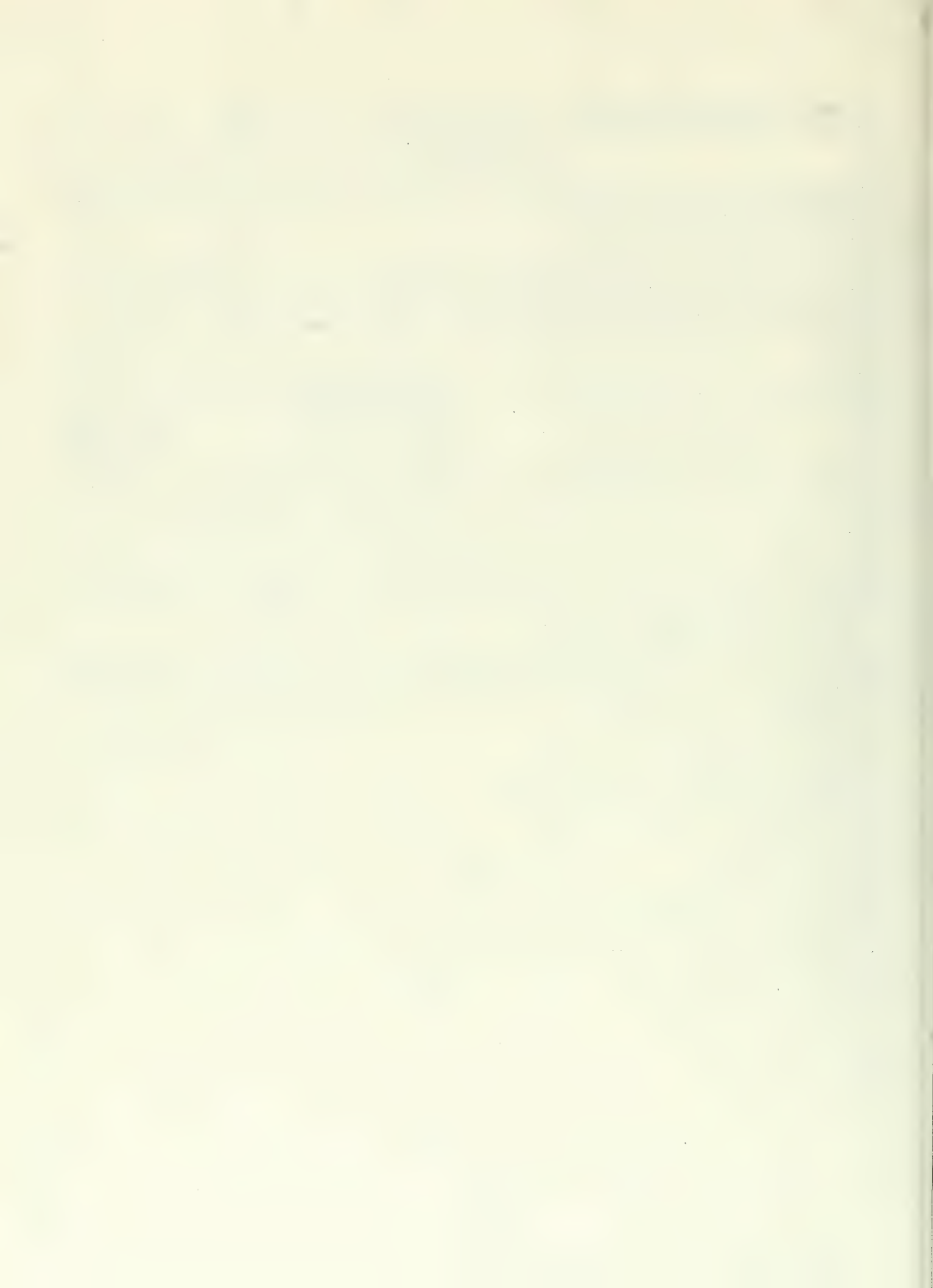
Significance to Mental Health Research: As the treatment of alcoholism has been relatively unsatisfactory in adult samples, the identification and possible prevention of high risk individuals assumes great significance. Alcoholism in adults is a major public health problem.

Major Findings: Blood acetaldehyde and breath acetaldehyde and breath alcohol reached peak at 30 minutes, but did not differ between groups. Clinically, children did not become overly intoxicated in spite of moderate alcohol dose used. Plasma epinephrine increased significantly with alcohol, while plasma cortisol decreased. These physiological measures did not predict behavioral response to ethanol. In contrast, baseline mood state did predict behavior 30 minutes post alcohol ingestion. Children feeling ill at baseline tended to become tired and less talkative; children feeling tired or sad at baseline tended to become more lively.

Proposed Course of Project: The skin biopsies are being conducted in Dr. Mukherje's laboratory. The alcohol and acetaldehyde dehydrogenase activities for the fibroblasts will be related to blood and breath acetaldehyde and to behavioral effects.

Publications: None to date.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00176-02 BP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Treatment of Hyperactive Children with Clorgyline | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| P.I.: Other: | Judith L. Rapoport, M.D. Alan Zametkin, M.D. Christy L. Ludlow, Ph.D. Dennis Murphy, M.D. Michael H. Ebert, M.D. | Chief, Unit on Childhood Mental Illness Clinical Associate Speech Pathologist Chief Chief, Section on Experi- Mental Therapeutics LCS NIMH NIMH |
| | | BP NIMH BP NIMH CDP NINCDS CN NIMH BP NIMH |
| COOPERATING UNITS (if any) Section on Experimental Therapeutics Clinical Neuropharmacology Branch | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Unit on Childhood Mental Illness | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 0.75 | OTHER: 0.25 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Projected terminated. | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00177-01 BP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Treatment of Hyperactive Children with Monoamine Oxidase Inhibitors | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| P.I.: Other: | Judith L. Rapoport, M.D. Alan Zametkin, M.D. Christy L. Ludlow, Ph.D. Dennis Murphy, M.D. Michael H. Ebert, M.D. | Chief, Unit on Childhood Mental Illness Clinical Associate Speech Pathologist Chief Chief, Section on Experi- mental Therapeutics |
| | | BP NIMH BP NIMH CDP NIMH CN NIMH BP NIMH |
| COOPERATING UNITS (if any) | | |
| Section on Experimental Therapeutics Clinical Neuropharmacology Branch | | LCS NIMH NIMH |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Unit on Childhood Mental Illness | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 0.75 | OTHER: 0.25 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>Hyperactive boys were treated with up to 15 mg/day of <u>clorgyline</u>, a selective Monoamine Oxidase A Inhibitor, or amphetamine (0.5/mg/kg). A second group was treated with <u>tranylcypromine</u> (Parnate) up to 15 mg/day. Behavioral measures include motor activity, vigilance and parent and teacher behavior ratings. Biological measures include urinary MHPG, PEA and platelet MAO. The aim of the study is to elucidate neurotransmitter mechanisms in amphetamine's efficacy in hyperactivity.</p> | | |

Project Description:

Objectives: To see if an MAO Inhibitor is an efficacious alternative to amphetamine treatment of hyperactivity. To elucidate the neurotransmitter mechanisms in amphetamine treatment of hyperactivity.

Methods Employed: Hyperactive boys were given amphetamine (0.5 mg/kg), placebo and clorgyline (up to 15 mg) using a double blind crossover design modified by a 2-week placebo washout period between active drugs. Urinary catecholamine metabolites and platelet MAO will be measured to see if they predict or reflect drug effects.

Major Findings: Six children have completed the 11-week clorgyline protocol to date. Clinical impression is that clorgyline is as effective as amphetamine in improved behavior and vigilance. There have been no adverse reactions to clorgyline. Following cessation of clorgyline at NIH, the protocol is being continued with tranlylcypromine (Parnate). Open studies are underway with 2 children to assess dose.

Significance to Mental Health Research: As hyperactivity may be a forerunner of adult sociopathy, alcoholism and schizophrenia, studies on the treatment and pathophysiology of hyperactive children have wide implications throughout the field of mental health research.

Proposed Course of Project: A total of 20 children will be studied on the protocol.

Publications: None to date.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00178-01 BP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Brain Structure and Function in Developmental Neuropsychiatric Disorders | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| P.I.: Judith M. Rumsey, M.D. Other: Judith L. Rapoport, M.D. Alan Mirsky, Ph.D. Alison Grimes, Ph.D. Daniel Weinberger, M.D. Martha Denckla, M.D. | Staff Fellow Unit on Childhood Mental Illness Chief, Unit on Childhood Mental Illness Chief Audiologist Staff Psychiatrist Chief, Section on Autism | BP NIMH BP NIMH LPP NIMH CC NIH AP NIMH DN NIHCDs |
| COOPERATING UNITS (if any) Laboratory of Psychology and Psychopathology | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Unit on Childhood Mental Illness | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.5 | PROFESSIONAL: 1.0 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Autistic subjects are being studied with CT scans, brainstem auditory evoked potentials, PET scans, neuropsychological testing, psychiatric interviews, and supplementary measures. Cortical evoked potentials and cerebral blood flow studies are planned. Other groups to be studied are learning disabled children, schizophrenic individuals, and siblings of autistic individuals.</u> | | |

Project Description:

Objectives. Ongoing studies are aimed at identifying neuroanatomical and neurophysiological abnormalities which characterize autism.

Methods: Methods include CT scans, brainstem auditory evoked potentials, PET scans, and neuropsychological testing.

Major Findings: Work in progress suggests that when careful attention is paid to technical factors, there is little evidence of brainstem dysfunction on auditory evoked potentials in contrast to earlier reports.

Most CT scans have been read clinically as normal; a few have shown dilated ventricles within the brain. Additional study with objective measurements is planned.

Preliminary PET scan results suggest heightened rates of glucose use in limbic structures and related regions. Neuropsychological testing suggests memory defects, residual auditory processing deficits, deficits in abstract and social reasoning, as well as high abilities in visuospatial skills.

Significance to Mental Health Research: Thus far, these studies are most compatible with the hypothesis that autistic symptomatology is associated with dysfunction of structures that lie deep within the cerebral hemispheres (limbic structures, basal ganglia), above the level of the brainstem.

Proposed Course of Project: Schizophrenic adults will be studied with neuropsychological testing as a contrast group for autistic subjects. Additional electrophysiological and cerebral blood flow studies of autistic, schizophrenic and learning disabled populations are planned.

Publications: None to date. There will be three presentations at scientific meetings later this summer and in the fall.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00034-12 BP | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;"><u>October 1, 1981 through September 30, 1982</u></p> | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Psychological and Physiological Correlates of the Average Evoked Response</p> | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">M. S. Buchsbaum, M.D.</td> <td style="width: 30%;">Chief, Section on Clinical Psychophysiology</td> <td style="width: 15%;">BP NIMH</td> </tr> <tr> <td>OTHER:</td> <td>R. Coppola, D.Sc.</td> <td>Senior Engineer Laboratory of Psychology</td> <td>LPP NIMH</td> </tr> </table> | | | PI: | M. S. Buchsbaum, M.D. | Chief, Section on Clinical Psychophysiology | BP NIMH | OTHER: | R. Coppola, D.Sc. | Senior Engineer Laboratory of Psychology | LPP NIMH |
| PI: | M. S. Buchsbaum, M.D. | Chief, Section on Clinical Psychophysiology | BP NIMH | | | | | | | |
| OTHER: | R. Coppola, D.Sc. | Senior Engineer Laboratory of Psychology | LPP NIMH | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">Laboratory of Psychology and Psychopathology George Washington University, Washington, D.C.</p> | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;"><u>Biological Psychiatry Branch</u></p> | | | | | | | | | | |
| SECTION <p style="text-align: center;"><u>Clinical Psychophysiology</u></p> | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;"><u>NIMH, ADAMHA, NIH Bethesda, Maryland 20205</u></p> | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;"><u>1.0</u></td> <td style="text-align: center;"><u>0.5</u></td> <td style="text-align: center;"><u>0.5</u></td> </tr> </table> | | | TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | <u>1.0</u> | <u>0.5</u> | <u>0.5</u> | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | |
| <u>1.0</u> | <u>0.5</u> | <u>0.5</u> | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) HUMAN SUBJECTS</td> <td><input type="checkbox"/> (b) HUMAN TISSUES</td> <td><input type="checkbox"/> (c) NEITHER</td> </tr> <tr> <td colspan="3"><input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS</td> </tr> </table> | | | <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input type="checkbox"/> (c) NEITHER | <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input type="checkbox"/> (c) NEITHER | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p style="text-align: center;"><u>Evoked potentials at varying intensities of stimulation are being recorded at 16 points on the left hemisphere and computer interpolated maps being developed.</u></p> | | | | | | | | | | |

The brain, the chief target organ for psychiatry, has up to now been studied through approaches that were necessarily indirect. Blood, urine, and cerebrospinal fluid, though valuable indicators of neurochemical and neuropharmacological activity, are removed in time and place from the disordered thought, and diluted by the products of both functional and dysfunctional systems. Biopsy studies, though valuable, are seldom available because they traumatize the brain, and in any case cannot reflect the enormous chemical and functional heterogeneity of brain areas. Autopsy studies, while of great scientific interest, cannot provide clinically useful diagnostic tests. Electrophysiological studies can track a perceptual event by milliseconds, but usually rely on a few standard electrode placements to assess arousal, habituation or other activity, and are less useful for studying a delimited gyrus.

A variety of innovative technologies can now make anatomic and functional features of the brain both visible and quantifiable. X-ray transmission tomography (CT Scans) can yield measures of ventricular size, sulcal atrophy and hemispheric asymmetry which are now being actively studied in the major psychoses.

Positron emission tomography (PET) is a versatile approach utilizing the mathematics of CT scanning to produce slice images of radioisotope locations, opening unlimited vistas of metabolic studies. Positron emitters such as carbon-11 or fluorine-18 can be used to tag glucose, amino acids, drugs or neurotransmitter precursors, and many other molecules. Quantitative studies of the brain, previously possible only in animals sacrificed and sectioned, can now be carried out in normal volunteers and patients.

If a large enough number of electrodes are used, EEG and evoked potentials can also become imaging instruments. Maps of the cortical surface can be constructed using interpolation methods: these reveal familiar anatomic contours and may parallel local metabolic patterns. Careful studies of topographic contours, detection of lead-to-lead redundancy, and work with very closely spaced electrodes all indicate that the application of 40 to 100 close electrodes can produce much better maps, and is now technically feasible. This entirely hazard-free technology can be further enhanced by combinations with PET, NMR, or other imaging techniques to provide a functional basis for interpretation. EEG mapping is not only risk free but can be repeated regularly and at a cost several orders of magnitude less than other imaging techniques.

In our schizophrenic patients (6 men and 2 women) who had PET Scans, EEG was recorded during both the PET Scan and during a separate session. Data from that separate session is presented here as analysis of PET data is still in progress. Normal controls (2 for each schizophrenic, 10 men and 6 women), 6 of whom were also in the PET study, serve as controls. We report here the effects for delta and alpha activity. Spectral analysis revealed higher delta activity in the frontal areas in the schizophrenics. This was confirmed by 2-way ANOVA with repeated measures (diagnostic group by lead analysis). A significant diagnostic group difference was found (4.7 microvolts in normals versus 5.6 microvolts in schizophrenics, averaged across leads; $F = 7.23$, degrees of freedom (df) = 1,22, $p < .01$), and the anteroposterior gradient was confirmed ($F = 7.9$, df = 1,22, $p < .01$), although

no significant difference in group topography was confirmed ($F = 1.7$, $p = \text{NS}$). Alpha activity across the whole head was higher in normals (8.7 microvolts) than in schizophrenics (5.1 microvolts). This main effect in mean, eyes-closed alpha was confirmed statistically ($F = 4.25$, $df = 1,22$, $p < .05$), as was a different topography (EEG lead by group interaction, $F = 5.29$, $df = 15,330$, $p < .001$; conservative $df = 1,22$, $p < .05$).

Our results show a parallel with previous work relating low blood flow to increased EEG activity. Our schizophrenics had diminished frontal glucose use (a correlate of lower blood flow) and increased frontal delta. Similarly, our schizophrenics had relatively higher occipital glucose use and lower occipital alpha activity, consistent with the alpha-EEG correlations of Jacquey et al (1980). It appears as if our schizophrenics had a regional EEG and glucose pattern at least superficially similar to normals with their eyes open.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

These studies represent an attempt to elucidate the normal and abnormal etiologies of the EEG and its relationship to the psychological development of normal and schizophrenic populations. Topographic illustrations of EEG activity will further our understanding of regional brain activity which in turn will aid in future hypothesis testing of the differences between normal and abnormal groups. A direct approach such as this can quantify differences in cortical surfaces and be combined with other imaging techniques (e.g. PET Scan) to enhance our understanding of the complexities of behavior.

Proposed Course

This work is being extended by matching topographic patterns of EEG and evoked potentials to topographic metabolic images collected during positron emission tomography.

Publications:

Buchsbaum, M.S.: Review of: van Praag, H.M., Lader, M.H., Rafaelsen, O.J., Sachar, E.J. (Eds.) Handbook of Biological Psychiatry, Part II: Brain Mechanisms and Abnormal Behavior-Psychophysiology. New York, Marcel Dekker 1980. Am. J. Psychiatry 138: 1644, 1980.

Buchsbaum, M.S. Brain imaging. Biol.Psychiatry, in press.

Buchsbaum, M.S., Cappelletti, J., Coppola, R., Rigal, F., King, A.C., and van Kammen, D.P.: New methods to determine the CNS effects of antigeriatric compounds: EEG topography and glucose use, in press.

Buchsbaum, M.S., Coppola, R., Gershon, E.S., van Kammen, D.P., and Nurnberger J.I.: Evoked potential measures of attention and psychopathology. Adv. Biol. Psychiatry 6: 186-194, 1981.

Buchsbaum, M.S., Haier, R.J., and Johnson, J.L.: Augmenting and reducing individual differences in evoked potentials. In Gale, A. and Edwards, J. (Eds.): Physiological Correlates of Human Behavior. London, Academic Press, in press.

Buchsbaum, M.S. and Johnson, J. L. Electrophysiological correlates of cognitive dysfunctions in psychiatric patients, including senile dementia. In Adam, G., Meszanos, Il, and Banyai, E.I. (Eds.): Advances in Physiological Science, Brain and Behavior. London, Pergamon Press, 1981, pp. 423-430.

Buchsbaum, M.S., King, A.C., Cappelletti, J., Coppola, R., and van Kammen, D.P.: Visual evoked potential topography in patients with schizophrenia and normal controls. Adv. Biol. Psychiatry, in press.

Buchsbaum, M.S., Rigal, F., Coppola, R., Cappelletti, J., King, C., and Johnson, J. A new system for gray-level surface distribution maps of electrical activity. Electroencephalogr. Clin. Neurophysiol. 53: 237-242, 1982.

Coppola, R., Buchsbaum, M.S., and Rigal, F. Computer generation of surface distribution maps of measures of brain activity. Comput. Bio. Med., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00035-10 BP |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 through September 30, 1982</p> | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Biochemical and Psychopharmacological Correlates of the Average Evoked Response</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | M. S. Buchsbaum | Chief, Section on Clinical Psychophysiology |
| | | BP NIMH |
| OTHER: | F. K. Goodwin D. L. Murphy T. P. Zahn R.D. Coursey R.J. Haier J. Irving | Chief Chief Research Psychologist University of Maryland Brown University Univ. Maryland |
| | | CP NIMH CN NIMH LPP NIMH Dept. Psychology Dept. Psychiatry Dept. Psychology |
| COOPERATING UNITS (if any) <p style="text-align: center;">Clinical Psychobiology Branch; Clinical Neuropharmacology Branch; Lab. of Psychology and Psychopathology; DCBR, NIMH. Dept. of Clinical Psychology, University of Maryland; Department of Psychiatry, University of Minnesota.</p> | | |
| LAB BRANCH <p style="text-align: center;">Biological Psychiatry Branch</p> | | |
| SECTION <p style="text-align: center;">Section on Clinical Psychophysiology</p> | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | |
| TOTAL MANYEARS: <p style="text-align: center;">1.8</p> | PROFESSIONAL: <p style="text-align: center;">0.6</p> | OTHER: <p style="text-align: center;">1.2</p> |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p style="text-align: center;"> <u>Evoked responses (EPs), platelet MAO, and urinary MHPG were studied in normal volunteers and patients with affective disorders. Relationships between EPs and these measures revealed important individual differences.</u> </p> | | |

Z01 MH 00035-10 BP

Cooperating Units

Department of Psychiatry, Brown University, Providence, Rhode Island.

Project Description

The initial excitement about the finding of low levels of platelet monoamine oxidase activity (MAO) in schizophrenia and its widespread replication has recently been tempered by reports that neuroleptics may lower MAO levels. The finding of low MAO in patients with bipolar affective disorder is also vulnerable to the criticism that excessive alcohol intake might similarly lower MAO. Studies in hospitalized patients, or drug abusing patients, even if off all medications at the time of sample collection, are always open to this methodological problem. Psychoactive medications are often administered for years in high doses, making brief off-medication periods a somewhat inadequate control. This is especially true for neuroleptics where clinical effects may persist for months after stopping the drug.

Screening for individuals in a non-hospitalized and non-chronically medicated community sample is a strategic adjunct to hospital-based studies which minimize these artifacts. A sample homogeneous with respect to the biological variable in question can be identified, interviewed, and followed.

In 1976, college-age probands were selected solely on the basis of extremely high or low MAO levels. In this study, low MAO subjects reported a two-fold higher incidence of seeing a psychiatrist or psychologist for personal problems. Three past psychiatric hospitalizations were found for the low MAO group (two suicide attempts and one depressive episode) and none in the high MAO group. These problems were more noticeable in the male than female probands. A history of psychiatric hospitalization, suicide, conviction or jail sentence for a legal offense was found in 10 of 19 male low MAO probands in comparison to 3 of 17 high MAO probands. Low MAO males were also more socially active, experimented more with drugs and had elevated scores on the sensation seeking scale. A similar pattern was found in their families. From information based on the probands reporting their first and second degree relatives, 10 of 17 probands with low MAO had at least one relative with psychiatric hospitalization, suicide or suicide attempts or problems with the law in contrast to 3 of 17 high MAO probands.

In a follow-up, subjects were briefly interviewed 2 years after completion of initial studies. The 33 low-MAO subjects reported more job instability than 30 high-MAO control subjects. Moreover, the low-MAO males had fallen about half a year behind their high-MAO counterparts in school. No difference in other aspects of social status or psychosocial problems had developed, although the low-MAO subjects smoked significantly more cigarettes and tended to report more major or minor medical problems. While the low-MAO subjects reported no significant decline in their own mental health status during this period, more low-MAO male subjects did report mental health problems in their families, especially depression, alcoholism, and suicide attempts, as well as significantly more mental health interventions among family members, such as psychiatric visits, prescription of psychotropic medication and psychiatric hospitalization.

In a second study, a sample of 241 men from suburban, rural, and small town social clubs participated in the study. Blood samples were drawn at evening club meetings and questionnaires, including a checklist from the Peri Life Events Inventory and the Michigan Alcohol Screening Test, were distributed. These were returned by 178 or 74% of the individuals.

As in the original, contact with a mental health professional (psychiatrist or psychologist) was significantly more frequent in low MAO probands (7 of 27 low MAO, vs. 1 of 26 medium, and 3 of 24 high MAO individuals, chi-square = 5.89, $p < 0.05$). Alcohol use was also associated with low MAO ("time since last drink" analysis showed significantly more low MAO probands in the "today or yesterday" category). Individuals with greater life stress were also more likely to have low MAO.

Proposed Course of Project

Continued analysis of community sample data; continued collection of MAO data on patients in PET Scan project.

Biomedical Significance

We have hypothesized elsewhere that low MAO may produce a generalized vulnerability to manifest psychopathology rather than any particular type. An example of such a phenomenon would be the appearance of depression, mania, or thought disorder following the hormonal upheaval of the post partum period. Low MAO being related to traits such as sensation seeking, disinhibition of impulse control, and/or rule breaking could call attention and concern to persons which would facilitate their entrance into the mental health system. Such an effect would more easily be revealed in a strategy which begins with low MAO probands than with individuals hospitalized for many reasons.

Lastly, the fact that neuroleptics appear to lower MAO levels is not necessarily indicative of the need to find a new marker. Studies in community samples have reported relationships between behavioral traits and MAO without intermediary neuroleptic effects. Indeed, if platelet MAO is an important marker of the central catecholamine economy, then a powerful drug might well be expected to alter it. A clearer delineation of how generalized risk factors interact with life stress and how secondary risk factors work in combination with MAO is the task for the community based epidemiological approach to the biology of psychopathology.

Publications

Buchsbaum, M.S. and Bunney, W.E. Jr.: Endorphins: Endogenous control of the perception of pain. In Gove, W.R. and Carpenter, G.R. (Eds.): The Fundamental Connection between Nature and Nurture. Lexington Books, Lexington, Massachusetts, 1982, pp. 41-55.

Buchsbaum, M.S. and Davis, G.C. Biological heterogeneity in schizophrenic patients: A comment on Checkley et al. American Journal of Psychiatry 136: 1618, 1979.

Buchsbaum, M.S., Muscettola, G., and Goodwin, F.K. Urinary MHPG, stress response, personality factors and somatosensory evoked potentials in normal subjects and patients with major affective disorders. Neuropsychobiology 7: 212-224, 1981.

Coursey, R.D. and Buchsbaum, M.S.: The biological high risk research strategy: A review of recent studies. In Regier, D. A. and Allen, G. (Eds.): Risk Factor Research in the Major Mental Disorders. Washington, D.C., Supt. of Docs., U.S. Government Printing Office, National Institute of Mental Health, DHHS Publ. No. (ADM)81-1068, 1981, pp. 163-176.

Huhtaniemi, P., Haier, R.J., Fedio, P., and Buchsbaum, M.S.: Neuropsychological characteristics of attention dysfunction in college males, in press.

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|---|--|--|-----|---|---------|--------|--|---------|--|---|---------|--|---|----------|--|---------------------------------------|---------|--|--------------------------------|--|--|--------------------------|----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00036-08 BP | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Individual Differences in Sleep and the AER</p> | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 55%;">M. S. Buchsbaum, M.D. Chief, Section on Clinical Psychophysiology</td> <td style="width: 30%;">BP NIMH</td> </tr> <tr> <td>OTHER:</td> <td>J. C. Gillin, M.D. Research Psychiatrist</td> <td>BP NIMH</td> </tr> <tr> <td></td> <td>R. M. Post, M.D. Acting Chief, Biological Psychiatry Branch</td> <td>BP NIMH</td> </tr> <tr> <td></td> <td>W. B. Mendelson, M.D. Research Psychiatrist</td> <td>SMR NIMH</td> </tr> <tr> <td></td> <td>W. Duncan, M.D. Research Psychiatrist</td> <td>BP NIMH</td> </tr> <tr> <td></td> <td>R. Coppola, D.Sc. Sr. Engineer</td> <td></td> </tr> <tr> <td></td> <td>Laboratory of Psychology</td> <td>LPP NIMH</td> </tr> </table> | | | PI: | M. S. Buchsbaum, M.D. Chief, Section on Clinical Psychophysiology | BP NIMH | OTHER: | J. C. Gillin, M.D. Research Psychiatrist | BP NIMH | | R. M. Post, M.D. Acting Chief, Biological Psychiatry Branch | BP NIMH | | W. B. Mendelson, M.D. Research Psychiatrist | SMR NIMH | | W. Duncan, M.D. Research Psychiatrist | BP NIMH | | R. Coppola, D.Sc. Sr. Engineer | | | Laboratory of Psychology | LPP NIMH |
| PI: | M. S. Buchsbaum, M.D. Chief, Section on Clinical Psychophysiology | BP NIMH | | | | | | | | | | | | | | | | | | | | | |
| OTHER: | J. C. Gillin, M.D. Research Psychiatrist | BP NIMH | | | | | | | | | | | | | | | | | | | | | |
| | R. M. Post, M.D. Acting Chief, Biological Psychiatry Branch | BP NIMH | | | | | | | | | | | | | | | | | | | | | |
| | W. B. Mendelson, M.D. Research Psychiatrist | SMR NIMH | | | | | | | | | | | | | | | | | | | | | |
| | W. Duncan, M.D. Research Psychiatrist | BP NIMH | | | | | | | | | | | | | | | | | | | | | |
| | R. Coppola, D.Sc. Sr. Engineer | | | | | | | | | | | | | | | | | | | | | | |
| | Laboratory of Psychology | LPP NIMH | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">Clinical Psychopharmacology, St. Elizabeth's Hospital, Washington, D.C.</p> | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Biological Psychiatry Branch</p> | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION <p style="text-align: center;">Clinical Psychophysiology</p> | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | | | | | | | |
| 0.3 | 0.1 | 0.2 | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | | | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | | |
| <p>New <u>techniques</u> for analyzing local <u>EEG processes</u> during <u>sleep stages</u> are under development. These include computer-generated cortical maps of power spectral estimates derived from 16 leads during sleep.</p> | | | | | | | | | | | | | | | | | | | | | | | |

Z01 MH 00036-08 BP

PROJECT DESCRIPTION

Computer-generated cortical maps of power spectral estimates derived from 16 leads were drawn based on daytime sleep recordings in four normal volunteers. These data were compiled from 9 ten-second, artifact-free, EEG epochs from awake, stages 1-4 and REM sleep in each volunteer. EEG leads were placed on the left hemisphere and midline according to the 10-20 system with 4 additional interpolated posterior locations. Magnitude spectral estimates with 1 Hz resolution and adjacent frequencies (delta 2-4, alpha 8-12, beta 13-18) were analyzed with 2-way ANOVA (lead by sleep stage). Delta activity was relatively uniform and of low amplitude in awake, eyes-closed subjects, and REM. Delta power increased at the vertex in stage 1. With progressing, non-REM sleep stages, it increased in power and enlarged radially to the intraparietal sulcus posteriorly, and the superior frontal gyrus anteriorly. Comparison of maps with ear and a computed average reference yielded similar topographic patterns.

Alpha activity was expectedly maximal occipitally in awake subjects, but surprisingly a frontal area appeared in slow wave sleep. Beta activity in awake subjects was low and maximal parietally; stages 1 and REM showed even lower and more uniform distribution. Stage 2 showed the greatest power, concentrated at the vertex, with stages 3 and 4 diminishing.

Proposed Course of Project

All night sleep records are currently being made in normal volunteers and patients with schizophrenia.

Significance to Biomedical Research and the Program of Institute

Sleep abnormalities are known in both affective disorders and schizophrenia. Specific topographic analysis may enable more accurate sleep staging and identification of individual cortical areas involved.

PUBLICATION:

Buchsbaum, M.S., Mendelson, W.B., Duncan, W.C., Coppola, R., Kelsoe, J. and Gillin, J.C.: Topographic cortical mapping of EEG sleep stages during daytime naps in normal subjects. Sleep, In press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00037-10 BP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Genetic Factors in Psychiatric Illness and the AER | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | M. S. Buchsbaum, M.D. Chief, Section on Clinical Psychophysiology | BP NIMH |
| OTHER: | E. S. Gershon, M.D. Chief, Section Psychogenetics J. I. Nurnberger Sect. on Psychogenetics R. Cromwell, M.D. University of Rochester R. D. Coursey, M.D. University of Maryland L. B. Purchall, M.D. University of Maryland X. O. Breakefield Ph.D. Associate Professor C. M. Castiglione, M.S. Associate in Research E. L. Giller, Jr., M.D. Associate Professor | BP NIMH BP NIMH Yale University Yale University Yale University |
| COOPERATING UNITS (if any) University of Rochester, Rochester, New York University of Maryland, College Park, Maryland | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Clinical Psychophysiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 0.4 | PROFESSIONAL: 0.2 | OTHER: 0.2 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been terminated. | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 00039-08 BP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Sensory Thresholds and Averaged Evoked Responses | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: M. S. Buchsbaum, M.D. OTHER: W. E. Bunney, Jr., M.D. G. C. Davis, M.D. F. K. Goodwin, M.D. R. M. Post, M.D. D. L. Murphy, M.D. M. Webster | Chief, Section on Clinical Psychophysiology Chief, Biological Psychiatry Branch Staff Psychiatrist Chief, Clinical Psychobiology Branch Chief, Section on Psychobiology Chief, Clinical Neuropharmacology Branch Research Assistant | BP NIMH BP NIMH University of Tennessee CP NIMH BP NIMH CNP NIMH CP NIMH |
| COOPERATING UNITS (if any) Clinical Neuropharmacology Branch; Clinical Psychobiology Branch, DCBR, NIMH. Department of Psychiatry, University of Tennessee, Memphis, Tennessee. | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Clinical Psychophysiology | | |
| INSTITUTE AND LOCATION NIMH, ADMAHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 0.9 | PROFESSIONAL: 0.3 | OTHER: 0.6 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Average evoked responses (AER) to varying intensities of stimulation to the forearm and subjective ratings of these stimuli have been studied in 155 age- and sex-matched normal volunteers. The effect of <u>analgesics</u> in normals and naltrexone in schizophrenics was also studied. | | |

PROJECT DESCRIPTION:

Pain and discomfort have long been known as the constant companions of psychiatric illness. Do patients with major affective illness and schizophrenia have a disturbed perceptual experience of pain as a result of their illness? What are the effects of major psychotropic drugs on pain? Are psychotropic drugs analgesics? In this era of neuropsychopharmacology, these questions can be more effectively approached than before. Many of the neuronal and behavioral actions of these drugs are now known and, in addition, we are beginning to understand the neuronal mechanisms for integration of pain. Thus, particular emphasis on the psychological aspects of pain in future research is desirable.

Disorders of attention are among the most prominent and consistent psychopathological findings in schizophrenia. Three lines of evidence suggest that opioid peptides may mediate disorders of attention in man: pain perception is dependent upon endorphinergic neuronal mechanisms and is intimately associated with attention; schizophrenics are relatively pain insensitive; opiates and opiate antagonists affect attentional performance and schizophrenic thought disorders may be modified by opiates and their antagonists. In an effort to extend previous studies of opioid peptides and related neurotransmitters, we have studied the influence of ACTH, morphine, and opioid antagonists on both attention and pain.

The EP technique provides several advantages in pain and schizophrenia research. In psychophysical tasks, whether reaction time or pain ratings, there is a whole series of neurophysiological events and stimulus responses by the motor system. Slow reaction time or low pain ratings could be the result of phenomena ranging from primary cortical atrophy to deficient social motivation or interfering delusions.

Careful use of EP technology may help isolate dysfunctional neural events. If two drugs or experimental manipulations affect the same EP component, then some communality of mechanism is more strongly suggested than would be by similar effects on a psychophysical task. With the EP technique, psychomotor performance is unnecessary, permitting the testing of catatonic, disturbed, paralyzed or unconscious patients. Further, not only have unmedicated schizophrenics been found to have impaired fine motor coordination, but abnormalities of the motor system itself, including pathological muscle fibers, have been reported, and EMG studies of schizophrenics' reaction time suggest that peripheral factors may be partly contributing to differences previously held to be due solely to central dysfunction.

Last year, studies of the detailed topographic distribution of evoked potentials was introduced (see project Z01 MH 00041-02 BP). The cortical topography of the somatosensory EP was studied in seven normal volunteers. With wrist stimulation, the peak at the top of the somatosensory cortex for the low intensity is expected. As stimulus intensity increases from levels normally reported as light touch to levels reported as unpleasant, pricking, and painful, the distribution spreads into posterior and superior parietal areas.

Six patients with schizophrenia (mean age 22, five males, one female) all off medication two weeks or more and 12 age- and sex-matched normal volunteers

PROPOSED COURSE OF PROJECT

BIOMEDICAL SIGNIFICANCE TO THE INSTITUTE

Publications

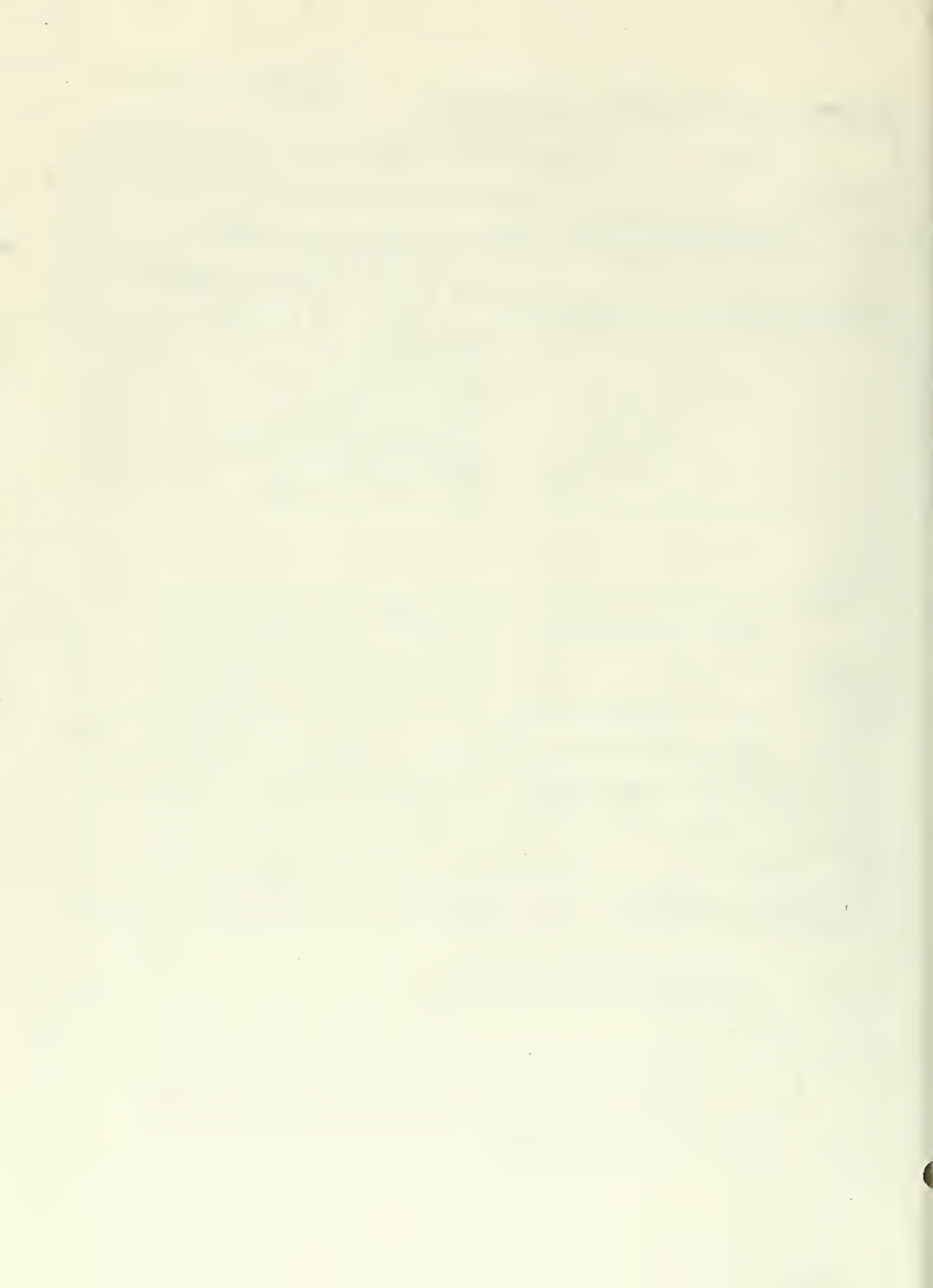
Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Opiates, opioid peptides and psychiatry. *Ann. N.Y. Acad. Sci.* 362: 67-75, 1981.

Davis, G.C., Buchsbaum, M.S., Naber, D., Pickar, D., Post, R., van Kammen, D., and Bunney, W.E. Jr.: Altered pain perception and CSF endorphins in psychiatric illness. N.Y. Aca. Sci. in press.

Dubois, M., Coppola, R., Buchsbaum, M.S., and Lees, D.E.: Somatosensory evoked potentials during whole body hyperthermia in humans. Electroencephalogr. Clin. Neurophysiol. 52: 157-162, 1981.

Rey, A.C., Buchsbaum, M.S., and Post, R.M.: Apomorphine, haloperidol and the average evoked potentials in normal human volunteers. Commun. Psychopharmacol. 4: 327-334, 1980.

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|--|--|---|-----------------|-----------------------|---|-----|------|-----|-------------------|---|-----|------|--|-------------------|---|----|------|--|----------------------|--|----|------|--|---------------------|--------------------------|--|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00040-07 B | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 through September 30, 1982</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Amphetamine Pharmacokinetics and the Average Evoked Responses in Hyperactive Children</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">M. S. Buchsbaum, M.D.</td> <td style="width: 40%;">Chief, Section on Clinical Psychophysiology</td> <td style="width: 10%;">BP</td> <td style="width: 10%;">NIMH</td> </tr> <tr> <td></td> <td>M. H. Ebert, M.D.</td> <td>Chief, Section on Experimental Therapeutics</td> <td>LCS</td> <td>NIMH</td> </tr> <tr> <td></td> <td>G. L. Brown, M.D.</td> <td>Medical Officer, Unit on Childhood Mental Illness</td> <td>BP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>J. L. Rapoport, M.D.</td> <td>Chief, Section on Childhood Mental Illness</td> <td>BP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>A. J. Sostek, Ph.D.</td> <td>St. Elizabeth's Hospital</td> <td></td> <td>NIMH</td> </tr> </table> | | | PI: | M. S. Buchsbaum, M.D. | Chief, Section on Clinical Psychophysiology | BP | NIMH | | M. H. Ebert, M.D. | Chief, Section on Experimental Therapeutics | LCS | NIMH | | G. L. Brown, M.D. | Medical Officer, Unit on Childhood Mental Illness | BP | NIMH | | J. L. Rapoport, M.D. | Chief, Section on Childhood Mental Illness | BP | NIMH | | A. J. Sostek, Ph.D. | St. Elizabeth's Hospital | | NIMH |
| PI: | M. S. Buchsbaum, M.D. | Chief, Section on Clinical Psychophysiology | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | M. H. Ebert, M.D. | Chief, Section on Experimental Therapeutics | LCS | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | G. L. Brown, M.D. | Medical Officer, Unit on Childhood Mental Illness | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | J. L. Rapoport, M.D. | Chief, Section on Childhood Mental Illness | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | A. J. Sostek, Ph.D. | St. Elizabeth's Hospital | | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">Laboratory of Clinical Studies, DCBR, NIMH St. Elizabeth's Hospital</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Biological Psychiatry Branch</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION <p style="text-align: center;">Clinical Psychophysiology</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">0.2</td> <td style="text-align: center;">0.1</td> <td style="text-align: center;">0.1</td> </tr> </table> | | | TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | 0.2 | 0.1 | 0.1 | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p style="text-align: center;">This project has been terminated.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |



Project Description

Studies of cerebral blood flow in man and animal studies using autoradiographs of (C14)-2-deoxyglucose (2DG) have indicated that local cerebral glucose use may parallel local functional activity and that blood flow and EEG frequency measures may be correlated. We have simultaneously investigated local glucose metabolism using 18F-2DG with PET and EEG frequency with 16 lead topographic mapping in eight unmedicated schizophrenics and six age- and sex-matched normal controls. Subjects sat in an acoustically treated darkened room with eyes closed for ten minutes prior to, and thirty minutes following, injection of 3 to 5 mCi 18F-2DG. Following uptake, seven to eight horizontal scans parallel to the CM line were made. Glucose uptake was expressed in micromoles glucose/100g tissue/min following the equation developed by Sokoloff. EEG recordings were made beginning one minute after injection of the isotope and continuing for thirty minutes using 12 standard 10/20 system points on the left hemisphere and midline, and four additional points between existing posterior leads. Ten-second EEG epochs were edited for artifacts and then analyzed using fast Fourier transform techniques.

PET Scans were treated digitally, with a 2.3-cm strip peeled off each slice and ratios to whole-slice activity computed. Patients with schizophrenia showed lower ratios in the frontal cortex, indicating relatively lower glucose use than normal control subjects; this was consistent with previously reported studies of regional cerebral blood flow. Patients also showed diminished ratios for a 2.3-cm square that was positioned over central gray-matter areas on the left but not on the right side. These findings were statistically confirmed with repeated measures analysis of variance on log transformed glucose use values from the Sokoloff equation. Using digital techniques, consecutive PET slices have the skull and skin layers peeled off with new computer techniques. Next, a 1 cm thick cortical strip is peeled off the slice, conformed to the lateral brain view, and values between strips interpolated. The result is two simultaneously obtained electrophysiological and metabolic lateral views of brain function displayed in gray scale values represented by dot density. The cortical glucose use value can be assessed directly under each scalp EEG electrode. In resting, eyes-closed, normal subjects we observed significant correlations between glucose use in the occipital region and alpha activity (-0.75) consistent with the interpretation of the alpha rhythm as indicative of an idling brain in subjects.

PET Scans were also obtained in the Genain Quadruplets. All four showed a similar glucose use pattern of low uptake frontally. The right central gray region (caudate, putamen, internal capsule) was higher than the left in 3 of the 4, also consistent with our group findings.

A second series of normal volunteers and patients with schizophrenia and affective disorders has been started. In these subjects our standard somatosensory stimulation used in pain studies was applied to the right forearm. The relative pain insensitivity of schizophrenics, reports of opiate antagonist reversal of schizophrenic symptoms, and the enhanced hyperfrontal blood flow observed by Ingvar in normals exposed to pain stimulation all suggested this approach.

Preliminary data analysis reveals increases in glucose use in contralateral postcentral cortex in normals, and continued evidence of a different frontal to occipital glucose use pattern in schizophrenics and normals.

Proposed Course of Project

The series of patients will be extended to provide comparison data for normals and the two diagnostic groups. Simultaneously collected somatosensory evoked potentials will be related to patterns of glucose use.

Biomedical Significance

It has never before been possible to assess the functional activity of structures in the center of the brain such as the caudate nucleus. These centers are very important areas for the metabolism of dopamine, the neurotransmitter affected by the neuroleptic drugs which are the major treatment for schizophrenia.

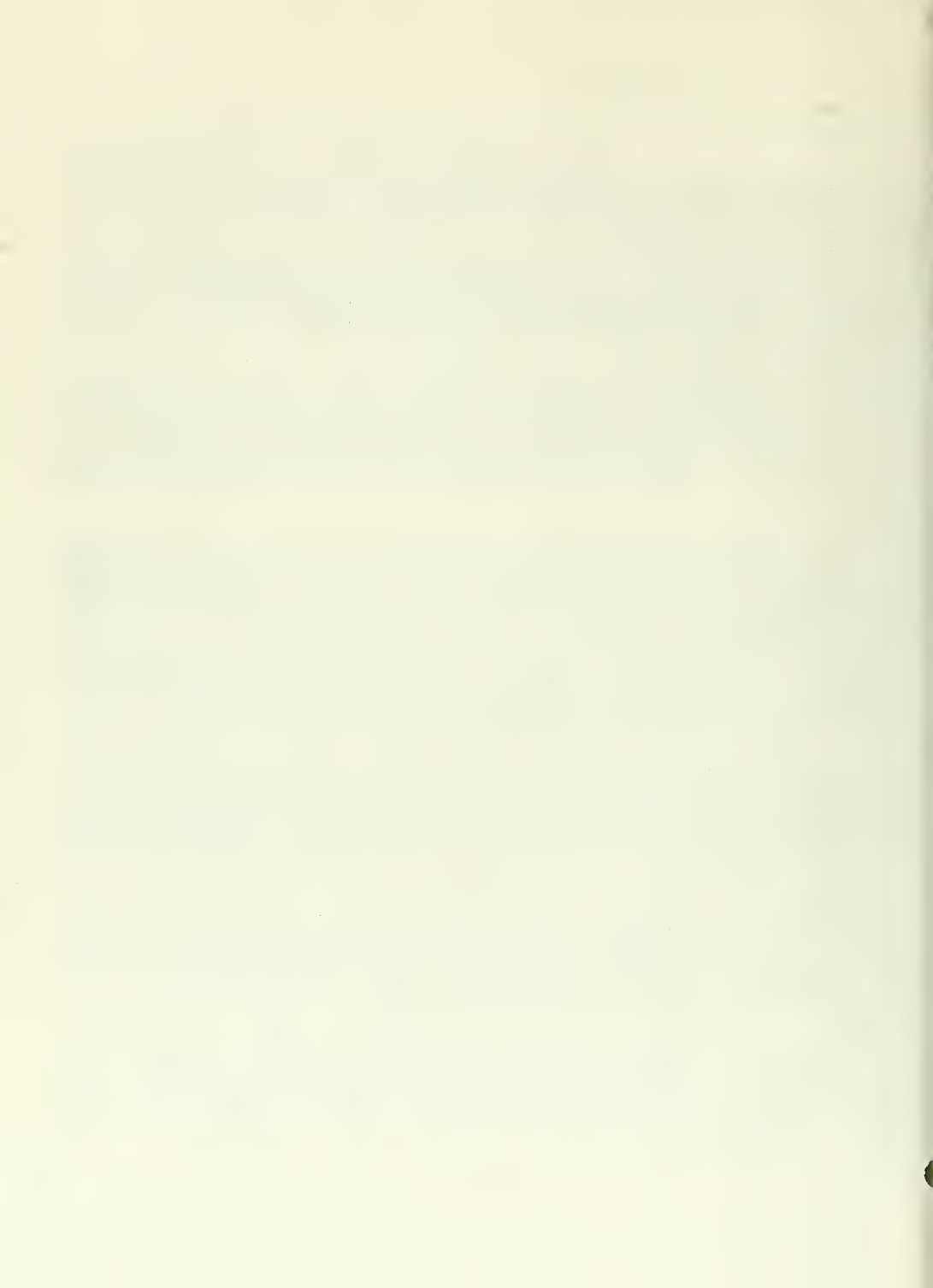
Publications

Buchsbaum, M.S., Coppola, R., and Cappelletti, J.: Positron emission tomography EEG and evoked potential topography: New approaches to local function in pharmaco-electroencephalography. In Herrmann, W.M. (Ed.): Electroencephalography in Drug Research. West Germany, Gustav Fischer Verlag, in press.

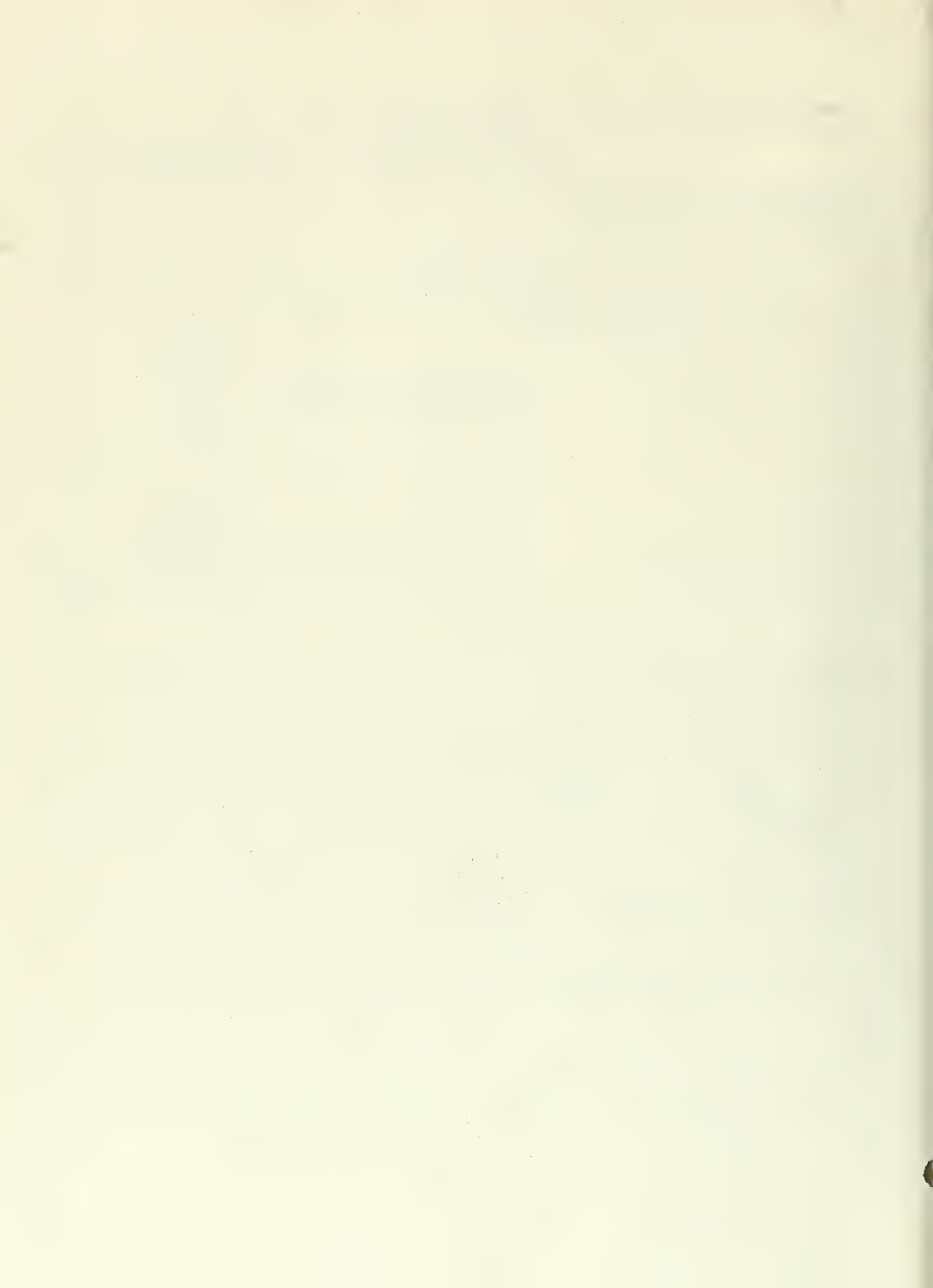
Buchsbaum, M.S. and Ingvar, D.: New visions of the schizophrenic brain: Regional differences in electrophysiology, blood flow and cerebral glucose use. In Henn, F.A. and Nasrallah, H.A. (Eds): Schizophrenia as a Brain Disease. New York, Oxford University Press, in press.

Buchsbaum, M.S., Ingvar, D.H., Kessler, R., Waters, R.N., Cappelletti, J., van Kammen, D.P., King, A.C., Johnson, J.L., Manning, R.G., Flynn, R.M., Mann, L.S., Bunney, W.E., Jr., and Sokoloff, L.: Cerebral glucography with positron tomography in normals and in patients with schizophrenia. Arch. Gen. Psychiatry 39: 251-259, 1982.

Buchsbaum, M.S., Kessler, R., Bunney, W.E., Jr., Cappelletti, J., Coppola, R., van Kammen, D.P., Rigal, F., Waters, R., Sokoloff, L., and Ingvar, D.: Simultaneous electroencephalography and cerebral glucography with positron emission tomography (PET) in normals and patients with schizophrenia. Journal of Cerebral Blood Flow and Metabolism 1, (Suppl. 1): 457-458, 1981.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 MH 00111-08 BP</div> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1980 to September 30, 1981 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Drug Abuse Studies/Stimulant Abuse/Opiate Abuse | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I. <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">William E. Bunney, Jr., M.D.</td> <td style="width: 40%;">Chief</td> <td style="width: 20%;">BP, NIMH</td> </tr> <tr> <td>David Pickar, M.D.</td> <td>Staff Psychiatrist, Unit on Studies of Drug Abuse</td> <td>BP, NIMH</td> </tr> <tr> <td>Martin R. Cohen, M.D.</td> <td>Guest Worker</td> <td>BP, NIMH</td> </tr> <tr> <td colspan="3">Other:</td> </tr> <tr> <td>Elliot S. Gershon, M.D.</td> <td>Chief, Section on Psychogenetics</td> <td>BP, NIMH</td> </tr> <tr> <td>Dieter Naber, M.D.</td> <td>Guest Worker</td> <td>BP, NIMH</td> </tr> <tr> <td>Irl Extein, M.D.</td> <td>Guest Worker</td> <td>CP, NIMH</td> </tr> <tr> <td>John Nurnberger, M.D.</td> <td>Staff Psychiatrist, Section on Psychogenetics</td> <td>BP, NIMH</td> </tr> <tr> <td>S.C. Schulz, M.D.</td> <td>Guest Worker</td> <td>BP, NIMH</td> </tr> </table> | | | William E. Bunney, Jr., M.D. | Chief | BP, NIMH | David Pickar, M.D. | Staff Psychiatrist, Unit on Studies of Drug Abuse | BP, NIMH | Martin R. Cohen, M.D. | Guest Worker | BP, NIMH | Other: | | | Elliot S. Gershon, M.D. | Chief, Section on Psychogenetics | BP, NIMH | Dieter Naber, M.D. | Guest Worker | BP, NIMH | Irl Extein, M.D. | Guest Worker | CP, NIMH | John Nurnberger, M.D. | Staff Psychiatrist, Section on Psychogenetics | BP, NIMH | S.C. Schulz, M.D. | Guest Worker | BP, NIMH |
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| S.C. Schulz, M.D. | Guest Worker | BP, NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Clinical Psychobiology Branch, DCBR, NIMH Section on Psychogenetics | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION <div style="text-align: center;">Unit on Studies of Drug Abuse, BP, NIMH</div> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NIMH, ADAMHA, Bethesda, MD 20205</div> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">2.25</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">1.0</div> | OTHER: <div style="text-align: center; font-weight: bold;">1.25</div> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) HUMAN SUBJECTS</td> <td><input type="checkbox"/> (b) HUMAN TISSUES</td> <td><input type="checkbox"/> (c) NEITHER</td> </tr> <tr> <td colspan="3"><input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS</td> </tr> </table> | | | <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input type="checkbox"/> (c) NEITHER | <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <div style="text-align: center; padding: 20px;"> <p style="font-size: 1.2em;">This project has been terminated.</p> </div> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 MH 00112-05 BP</div> |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center; font-weight: bold;">Endorphin Research in Mental Illness</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I.: <div style="display: flex; justify-content: space-between;"> <div>David Pickar, M.D.</div> <div>Staff Psychiatrist, Chief, Unit on Studies of Drug Abuse</div> <div>BP, NIMH</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>William E. Bunney, Jr., M.D.</div> <div></div> <div></div> </div> Other: <div style="display: flex; justify-content: space-between;"> <div>Robert M. Post, M.D.</div> <div>Chief, Section on Psychobiology</div> <div>BP, NIMH</div> </div> <div style="display: flex; justify-content: space-between;"> <div>Daniel P. van Kammen, M.D.</div> <div>Unit Chief, Section on Neuro-psychopharmacology</div> <div>BP, NIMH</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>Dieter Naber, M.D.</div> <div>Guest Worker</div> <div>BP, NIMH</div> </div> <div style="display: flex; justify-content: space-between;"> <div>David Rubinow, M.D.</div> <div>Staff Psychiatrist, Section on Psychobiology</div> <div>BP, NIMH</div> </div> | | |
| COOPERATING UNITS (if any) Clinical Psychobiology Branch, DCBR, NIMH Division of Special Mental Health Research, NIMH | | |
| LAB/BRANCH Biological Psychiatry Branch SECTION Unit on Studies of Drug Abuse | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">2.75</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">1.0</div> | OTHER: <div style="text-align: center; font-weight: bold;">1.75</div> |
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| SUMMARY OF WORK (200 words or less - underline keywords) <p> Since the discovery of the opiate receptor and <u>endogenous opioid ligands</u> (endorphins), there has been considerable speculation regarding the role of this <u>endogenous opioid system</u> in behavior and mental illness. The major goal of this project is to use available clinical research strategies to study endorphins in human behavior and <u>psychiatric illness</u>. The Unit has tested the behavioral effects of intravenously administered naloxone, a pure narcotic antagonist, and of <u>beta-endorphin</u>, an endogenous opioid peptide, in both <u>affectively ill</u> and <u>schizophrenic patients</u>. A sensitive radioreceptor assay used to determine <u>opioid activity</u> in human CSF and plasma has been developed and used in conjunction with <u>radioimmunoassay</u> for <u>beta-endorphin</u> to study endogenous opioid function in medication-free and drug treated psychiatric patients. We have now completed a <u>high dose-response naloxone</u> study in normal volunteers. Results indicate that considerably higher naloxone doses than previously thought are needed to block the endogenous opioid system. High dose naloxone effects support hypothesized roles for the endogenous opioid system in mood and physiologic regulation. </p> | | |

Names, Laboratory and Institute Affiliations Continued:

| | | |
|---------------------------------|--|------------|
| Robert Waters, M.D. | Section on Neuropsychopharmacology | BP, NIMH |
| Glenn C. Davis, M.D. | Guest Worker | BP, NIMH |
| Candace Pert, Ph.D | Section on Biochemistry | BP, NIMH |
| Martin R. Cohen, M.D. | Guest Worker | BP, NIMH |
| Robert M. Cohen, M.D., Ph.D. | Guest Worker | BP, NIMH |
| Fredrick K. Goodwin, M.D. | Chief | CP, NIMH |
| Richard Wagner, M.D. | Staff Psychiatrist, Laboratory of Clinical Psychopharmacology | DSMR, NIMH |
| Walter Kaye, M.D. | Staff Psychiatrist | LCS, NIMH |

Cooperating Units Continued:

Section on Psychobiology, BP, DCBR, NIMH
 Section on Neuropsychopharmacology, BP, DCBR, NIMH

Objectives:

The major objective of this project is to seek understanding of the role of endorphins in behavior and mental illness.

Methods Employed:

The following strategies have been used to study the role of endorphins in psychiatric patients.

1. The administration of opiate antagonists to psychotic patients. Depressed and schizophrenic patients from the Clinical Center and St. Elizabeth's Hospital have been administered with double-blind methodology the narcotic antagonist, naloxone, accompanied by careful clinical ratings to delineate possible behavioral effects of this drug. The Unit has served as the coordinating center for a World Health Organization Collaborative Project studying the effects of naloxone in schizophrenia and mania and is currently participating in a phase II study examining the therapeutic efficacy of a daily naloxone administration for a week's period.

2. High dose naloxone administration to normal volunteers. A dose-response study of high dose naloxone (up to 6 mg/kg) was performed in normal volunteers. This study included assessment of behavioral, physiologic and neuroendocrine parameters.

3. Assay of endogenous opioids in CSF. A sensitive radioreceptor assay to determine levels of opioid activity in CSF has been developed and used in conjunction with beta-endorphin immunoassay to study endogenous opioid activity in psychiatric patients. Levels of opioids have been examined with regard to behavioral, pharmacologic and biologic parameters.

Major Findings:

1. The Unit contributed to and coordinated the World Health Organization Collaborative Project, The Effect of Acute Naloxone Administration in Schizophrenic and Manic Patients. Results from this study showed that schizophrenic patients concurrently treated with neuroleptic medication showed significant improvement in overall symptomatology, including auditory hallucinations, while unmedicated schizophrenic patients showed no differences from placebo. No significant naloxone effects were observed in manic patients.

2. The Unit carried out a new project intended to examine dose-response effects of high doses of naloxone in normal volunteers. High naloxone doses produced clinically apparent behavioral changes including increases in anxiety, dysphoria and loss of appetite. Significant dose-response naloxone effects on mood self-ratings, blood pressure, cortisol and growth hormone suggested progressive opiate antagonist effects with increases in naloxone doses. These data suggest that larger naloxone doses than had previously been thought may be required to produce opiate receptor blockade. The data also supports the hypothesized involvement of the endogenous opioid system in mood regulation in normals and in important physiological and neuroendocrine systems.

3. Prior to clinical applications, the radioreceptor assay technique was applied to CSF samples from non-human primates. Opioid activity determined in four rhesus monkeys every two hours over twenty-four hours revealed longitudinal variation in opioid activity suggestive of episodic secretion and a significant diurnal rhythm with increased morning levels of opioid activity in comparison to afternoon levels. This observed diurnal rhythm is similar to that reported of beta-lipotropin and ACTH. Furthermore, it supports a need for careful control for time of CSF sampling in clinical studies.

CSF opioid activity was determined in 89 medication-free psychiatric patients and in 41 medication-free normal volunteers studied under controlled conditions on three nursing units (3-West, 4-West and 4-East). A significant decrease in opioid activity was found among schizophrenics as a whole compared to normals, although this difference was attributed to a rather marked deficit in opioid activity by the male schizophrenics in comparison with male controls; female schizophrenics were found to have comparable CSF opioid activity to female controls; no differences in CSF opioid activity were found between depressed subjects and normals including analysis by sex. In a subgroup of these patients, beta-endorphin immunoreactivity was determined. There were no differences in CSF beta-endorphin immunoreactivity between any diagnostic group and normal controls, including sex analysis, suggesting that the observed deficit in CSF opioid activity between male schizophrenics and male normals was related to opioid(s) other than beta-endorphin.

CSF opioid activity in the depressed patients, but not in normals, was significantly correlated with urinary free cortisol excretion. These data suggest that the observed activation of the hypothalamic-pituitary-adrenal axis in depression can be related to opioid activity. In a subgroup of depressed

patients, who were treated with the anticonvulsant and antidepressant drug, carbamazepine, pre-treatment CSF opioid activity significantly predicted response to drug treatment.

CSF samples from six patients with anorexia nervosa were assayed for opioid activity when patients were at minimal weight and at maximal weight following refeeding. CSF opioid activity was significantly elevated during the period of minimal weight in comparison to maximal weight; this increased opioid activity was significantly greater than that found in normal female controls, while levels when the subjects were refed were comparable to normal levels.

Significance to Biomedical Research:

1. Results from the World Health Organization Collaborative Project are relevant to research in endorphins and schizophrenia. The finding that concurrent neuroleptic treatment was associated with a positive response to naloxone suggests a possibility of a synergistic effect between chronic dopamine blockade by neuroleptics and acute endorphin blockade by naloxone. Such a relationship between the endogenous opioid system and dopamine system is consistent with a considerable amount of basic research. Further studies in this area are needed to delineate these effects as to clinical significance.

2. The results from our study of high dose naloxone provided important support for the hypothesized relationship of the endogenous opioid system to human behavior. This project also contributes to the important methodologic issue, i.e., that considerably larger doses of naloxone are needed to block the endogenous opioid system than had previously been thought.

3. The finding of a diurnal rhythm in CSF opioid activity in primates is consistent with other data regarding ACTH and other related peptides. This is the first such finding reported in the literature. We found no indication of elevations in CSF opioid activity among schizophrenic patients - this is in contrast to early speculation regarding increased endorphin activity in schizophrenia. Our findings relating CSF opioid activity to urinary free cortisol in depressed patients may contribute to further understanding of abnormalities in cortisol regulation in depression. The state-related changes in opioid activity in patients with anorexia nervosa contribute to accumulating evidence suggesting a role for endogenous opioids in eating behavior.

Proposed Course:

1. A Phase II Naloxone Project coordinated by the Unit for the WHO in which neuroleptic treated patients will be administered naloxone for four consecutive days is currently underway. This may aid in evaluating the clinical significance of the initial findings.

2. Continued analysis of CSF opioid data already generated in this project is currently in progress. This includes relating CSF opioid activity to a variety of specific symptomatology within diagnostic groups. Further research in this area will attempt to delineate which of the various endogenous opioids may be deficient in schizophrenic patients.

3. The Unit is in the final planning stages of the intrathecal administration of β -endorphin to patients with severe chronic pain. This project will enable evaluation of both the analgesic and behavioral effects of β -endorphin when administered with direct access to the CNS.

Publications:

Naber, D., Pickar, D., Davis, G., Cohen, R.M., Jimerson, D., Elchisak, M., Defraites, E., Kalin, N., Risch, C., and Buchsbaum, M.: Naloxone effects on β -endorphin, cortisol, prolactin, growth hormone, HVA and MHPG in plasma of normal volunteers. Psychopharmacology 74: 125-128, 1981.

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Pickar, D., Naber, D., Post, R.M., van Kammen, D.P., Ballenger, J.C., Waters, R.N., Rubinow, D., Goodwin, F.K., and Bunney, W.E., Jr.: CSF endorphins in psychiatric patients. Psychopharm. Bull. 17: 75-78, 1981.

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Naber, D., Pickar, D., Post, R.M., van Kammen, D.P., Ballenger, J.C., Goodwin, F.K., and Bunney, W.E., Jr.: Endogenous opioid activity and β -endorphin unreactivity in CSF of psychiatric patients and normal volunteers. Am. J. Psychiatry 38: 1457-1462, 1981.

Cohen, M.R., Nurnberger, J.I., Jr., Pickar, D., Gershon, E.S., and Bunney, W.E., Jr.: Dextroamphetamine infusions in normals results in correlated increases of plasma beta-endorphin and cortisol immunoreactivity. Life Sci. 29: 1243-1247, 1981.

Pickar, D., Naber, D., Post, R.M., van Kammen, D.P., Ballenger, J.C., and Bunney, W.E., Jr: Measurement of endorphins in CSF: Relationship to psychiatric diagnosis. In Emrich, H.M. (Ed.): Modern Problems in Pharmacotherapy: The Role of Endorphins in Neuropsychiatry. Basel, A.G. Karger, pp. 246-262, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00114-02 BP | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Surgical Stress and the Endogenous Opioid System | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | |
| <p>P.I.:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">David Pickar, M.D.</td> <td style="width: 40%;">Staff Psychiatrist, Chief, Unit on Studies of Drug Abuse</td> <td style="width: 25%;">BP, NIMH</td> </tr> <tr> <td>Michel DuBois, M.D.</td> <td>Section of Anesthesiology (Clinical Center)</td> <td>CC, NIMH</td> </tr> <tr> <td>Martin R. Cohen, M.D.</td> <td>Guest Worker</td> <td>BP, NIMH</td> </tr> <tr> <td colspan="3">Other:</td> </tr> <tr> <td>Yolanda Roth, M.D.</td> <td>Medical Oncology</td> <td>NCI</td> </tr> <tr> <td>William E. Bunney, Jr., M.D.</td> <td></td> <td>BP, NIMH</td> </tr> </table> | | | David Pickar, M.D. | Staff Psychiatrist, Chief, Unit on Studies of Drug Abuse | BP, NIMH | Michel DuBois, M.D. | Section of Anesthesiology (Clinical Center) | CC, NIMH | Martin R. Cohen, M.D. | Guest Worker | BP, NIMH | Other: | | | Yolanda Roth, M.D. | Medical Oncology | NCI | William E. Bunney, Jr., M.D. | | BP, NIMH |
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| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | |
| SECTION Unit on Studies of Drug Abuse | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">2.25</div> | PROFESSIONAL: <div style="text-align: center;">1.0</div> | OTHER: <div style="text-align: center;">1.25</div> | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | |
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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00070-09 BP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Psychological and Biological Interactions in the Acute Psychoses | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| P.I. R.M. Post, M.D. OTHER: T.W. Uhde, M.D. D.R. Rubinow, M.D. F.W. Putnam, M.D. W.H. Berrettini, M.D. C.H. Kellner, M.D. B. Scupi K.S. Bell H.A. Meyersburg, M.D. E. Silber, M.D. | Acting Chief Ward Chief, Sect. on Psychobiology Staff Psychiatrist Staff Psychiatrist Clinical Associate Clinical Associate Clinical Social Worker Clinical Social Worker Consultant Consultant | BP NIMH BP NIMH BP NIMH BP NIMH BP NIMH BP NIMH BP NIMH BP NIMH BP NIMH BP NIMH |
| COOPERATING UNITS (if any) Nursing Department, 3-West NIH Clinical Center Rehabilitation NIH | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Psychobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 12.0 | PROFESSIONAL: 6.0 | OTHER: 6.0 |
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| SUMMARY OF WORK (200 words or less - underline keywords) Patients suffering from <u>manic, depressive, schizoaffective, and anxiety-related</u> disorders are longitudinally evaluated and treated. <u>Double-blind, placebo-controlled</u> clinical trials are employed. Classical <u>neurotransmitters</u> and their metabolites, as well as <u>hormones</u> and <u>peptides</u> that have been implicated in the regulation of mood and behavior, are measured in blood and CSF of patients to assess their relationship to normal and pathological behavior. Alterations in cognitive function, neurophysiology, and biochemistry are explored in relationship to predictors and mechanisms underlying clinical response to <u>anticonvulsants, dopaminergic and noradrenergic receptor agonists</u> , the paradoxical therapeutic effects of <u>sleep deprivation</u> in depression, and related treatments of mood and anxiety disorders. Ongoing clinical trials with <u>carbamazepine</u> indicate it may be a useful alternative to lithium carbonate for the acute and prophylactic treatment of manic-depressive illness. Its mechanisms of action in affective illness are being explored. Animal models of <u>electrical kindling</u> and stimulant-induced <u>behavioral sensitization</u> are explored in order to examine mechanisms underlying progressive changes in behavioral pathology. | | |

Collaborators and Affiliations (cont'd)

| | | |
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| J. Patel | Visiting Fellow | CP NIMH |

I. Project Description

A. Objectives

This project is engaged in the multidisciplinary longitudinal study and treatment of patients with a spectrum of acute and chronic psychoses, particularly involving mood and anxiety disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interaction.

B. Methods Employed

1. Subjects

a. Subjects who meet Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness or the more recent DSM III criteria for a spectrum of mood disorders are admitted to the 3-West Clinical Research Unit, Section on Psychobiology. Patients with anxiety, panic anxiety, and phobic disorders, as well as those with multiple personality syndrome, are also admitted to the unit under other protocols (see Project #Z01 MH 00071-02 BP and #Z01 MH 00072-02 BP). Depressed patients are rated for degree of typicality of depression on a formal rating scale developed in collaboration with Dr. E. K. Silberman.

b. Normal volunteers are also admitted to the unit to provide control data for specific studies in patients and to assess clinical and biological interrelationships in normal as well as patient populations. Volunteers complete an extensive battery of biochemical, psychological, and physiological tests including lumbar punctures (LP's). LP's are performed at 9:00 a.m. and 9:00 p.m. to study alterations in circadian rhythms in patients and volunteers.

2. Psychological and Biological Evaluation

a. Behavior and Cognition. During an initial drug-free interval patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation, including EEG-monitored sleep, averaged evoked potentials, and a variety of cognitive tests. These include the Halstead Categories Test, the Levine Test, the cognitive style questionnaire, a psychosensory questionnaire, and a neuropsychological profile designed to assess disturbances in brain function with the Luria Battery. Studies of specific disturbances in memory and learning are conducted with Drs. D. R. Rubinow, J. P. Boulenger, H. Weingartner, and E. K. Silberman.

Longitudinal behavioral data are collected in a double-blind fashion utilizing twice-daily global ratings by trained nursing observers. Patients also complete twice-daily ratings of mood and side effects in order to examine diurnal variation. Using the same double-blind methodology, nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) three times weekly.

b. Life Chart Methodology. A life chart technique has been developed to plot the number and severity of affective episodes and the interval between episodes so that the longitudinal development, recurrence, and pro-

gression of the illness can be accurately quantitated and illustrated. This technique is an important clinical as well as research tool for assessing the efficacy of treatment interventions.

c. Physiology. Motor activity is measured continuously at 15-minute intervals with a miniaturized activity monitor developed by Dr. T. Colburn. EEG-monitored sleep is studied in collaboration with Dr. J. C. Gillin and W. Duncan. In collaboration with Dr. M. S. Buchsbaum, 16 channel EEGs, average evoked responses, and studies of hemispherical laterality and psychophysiological pain are conducted.

d. Functional Anatomy. In addition to computerized axial tomography (CAT) scan evaluation of our patients for possible cerebral pathology, studies have been initiated in collaboration with Dr. M. S. Buchsbaum and a large number of associates to study regional functional activity of the brain using (18F) flurodeoxyglucose.

e. An Alpha-Adrenergic Agonist, Clonidine. Clonidine is administered intravenously to depressed and anxious patients and volunteers in order to assess clinical, physiological, and neuroendocrine responses to this alpha-adrenergic agonist (with Dr T. W. Uhde in collaboration with Drs. L. Siever and D. L. Murphy).

f. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function, as well as prostaglandin-stimulated increases in cyclic AMP, are assessed in normal volunteers and patients with mood and anxiety disorders.

g. Urinary MHPG and Urinary Free Cortisol. These substances are evaluated in basal 24-hour urinary collections during depressed and manic states in medication-free conditions and during treatment.

h. Cerebrospinal Fluid (CSF) and Plasma Studies. Plasma and CSF studies compose a core area of biological evaluation of classical neurotransmitters and their amines as well as the newly discovered peptide substances in normal volunteers and in patients during ill and well intervals. These studies are conducted in collaboration with Drs. F. K. Goodwin, P. W. Gold, D. C. Jimerson, and M. H. Ebert, as well as many investigators within and outside of NIMH with specialized techniques for measurement of specific peptide hormones.

i. Oxytocin and Vasopressin. In collaboration with Drs. H. Weingartner, P. W. Gold, and Dr. D. R. Rubinow, infusions of these peptides are utilized to assess effects on memory, mood, and endocrine function in affectively ill patients and normal volunteers.

j. Dexamethasone Suppression. A detailed evaluation of the pituitary adrenal axis is conducted by Dr. D. R. Rubinow in patients with affective illness and anxiety disorders. Plasma and urinary free cortisol are measured under basal conditions and following the dexamethasone suppression test.

Cortisol receptor binding on lymphocytes is assessed in collaboration with Dr. D. C. Jimerson.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and group therapy, and ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. The routinely used drugs include tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics. These agents are utilized not only because of their clinical efficacy, but as well to further understand their mechanisms of action and possible interaction with the pathophysiology of the illness.

c. Experimental Compounds. The anticonvulsant carbamazepine has been introduced as a new treatment for manic and depressive illness and is evaluated for its acute and prophylactic efficacy. Diphenylhydantoin and valproic acid are two other anticonvulsant agents also being studied in selected patients to assess the specificity of the positive psychotropic effects of carbamazepine in relation to other anticonvulsants with different spectrums of clinical efficacy.

d. Receptor Agonists and Antagonists. The dopamine receptor agonist piribedil and the dopamine antagonist pimozide are utilized to study therapeutic response in relation to alterations in dopaminergic systems. Clonidine, in addition to acute intravenous studies, is administered during clinical trials in order to assess the clinical efficacy of alterations in adrenergic functioning in anxiety and affective illness.

e. Peptide Strategies. In addition to acute challenges with oxytocin and vasopressin, clinical trials have been conducted in collaboration with Dr. P. W. Gold of a vasopressin analog, DDAVP, in affective illness.

f. Sleep Deprivation. The paradoxical antidepressant effects of one night's sleep deprivation in depressed patients are explored both to develop a model for further understanding the rapid onset and offset of a non-pharmacologically-induced mood improvement and to assess its therapeutic potential.

g. Animal Models. A rodent and primate behavioral pharmacology laboratory is maintained to develop new research techniques in several areas. The longitudinal evolution of behavioral pathology is assessed using a number of paradigms including: 1) electrophysiological kindling; 2) pharmacological kindling; 3) behavioral sensitization to psychomotor stimulants and related dopaminergic agonist compounds; and 4) the evaluation of stress sensitization and its possible underlying neural substrates. Physiological and biochemical changes, in particular alterations in receptor binding, are studied in collaboration with Drs. M. Del Zompo, J. Tallman, and A. Pert. ¹⁴C-2-deoxyglucose studies have been conducted utilizing pharmacological kindling with lidocaine in collaboration with Drs. C. Kennedy, L. Sokoloff, and associates. The role of

seizures in the development of psychopathology is studied utilizing a variety of seizure models, behavioral assessment, and anticonvulsant compounds.

C. Major Findings

1. Carbamazepine: A New Treatment for Manic-Depressive Illness

a. Introduction. Several empirical observations and theoretical perspectives led to our initiation of the first double-blind, placebo-controlled clinical trials of carbamazepine in mania and depression in the United States. There had been persistent reports of positive effects on mood and behavior in epileptic patients treated with carbamazepine. Carbamazepine, both clinically and in experimental models such as kindling, is the most effective anticonvulsant against temporal lobe-limbic seizures. Temporal lobe and limbic structures have long been hypothesized to be importantly involved in the modulation of normal and pathological affect. As such, an agent which might stabilize abnormal excitability in this area of brain might be expected to have stabilizing effects on emotional function. Moreover, preliminary data from open clinical trials in Japan suggested that carbamazepine might be effective in manic-depressive patients when it was added to previously ineffective drug regimens.

b. Acute Antimanic Efficacy. We continue to document unequivocal evidence of the efficacy of carbamazepine in the acute treatment of manic episodes. It is noteworthy that this occurs in some patients who were previously non-responsive to lithium carbonate and neuroleptics, the more traditional agents for the treatment of affective illness. Evidence of carbamazepine response has been documented during an "off-on-off-on" design, where carbamazepine and placebo are administered in an alternate fashion, with nurses blind to this clinical trial. We have noted dramatic clinical improvement during carbamazepine treatment and exacerbation during placebo substitution.

c. Acute Antidepressant Efficacy. In the first 25 patients treated, 48% have shown evidence of clinical response to carbamazepine. In some instances, marked clinical improvement was observed, although relapses following placebo substitution were not as consistently observed in the depressive phase as they were in the use of carbamazepine for the treatment of mania. Therefore, while carbamazepine appears to have antidepressant properties in some depressed patients, further work remains to document the degree of clinical response and whether there are clinical and biological markers.

d. Effects on Sleep and Side Effects. The drug is well-tolerated in the majority of patients with mild and clinically insignificant decreases in white count observed in the majority of patients. Mild decreases in serum sodium are also observed. Sedation and dizziness are dose related and tend not to occur with slow increases in dose. Analysis of self ratings of side effects indicates that depressed patients experience a moderate incidence of apparent drug-related side effects while medication-free, and some "side effects" such as poor appetite or tiredness are significantly decreased during carbamazepine treatment.

Substantial improvement in sleep has been noted from half-hour sleep checks by nurses blind to active carbamazepine administration. In the first 27 de-

pressed patients, sleep significantly increased ($p < .001$) during the first week of carbamazepine and this improvement was maintained during the clinical trial. This improvement in sleep occurred without notable increases in daytime sedation. Similarly, in the first 11 manic patients studied, sleep almost doubled in the first week of carbamazepine administration ($p < .001$). Studies in collaboration with Dr. J. C. Gillin indicate that the improvement in sleep is occurring predominantly in stages 3 and 4 of EEG-monitored sleep.

e. Blood and CSF Levels of Carbamazepine and Its Active -10,11-epoxide metabolite: Relationship to Clinical Response. Spinal fluid levels of carbamazepine and its 10,11-epoxide metabolite were measured in 18 affectively ill patients. These studies were performed in collaboration with Drs. T. W. Uhde, D. C. Chatterji, and R. F. Greene. Mean CSF carbamazepine was 2.06 ± 0.10 $\mu\text{g/ml}$, while the epoxide was 0.91 ± 0.09 $\mu\text{g/ml}$ or 44% of the concentration of the parent compound. Carbamazepine levels in plasma or in CSF (a measure of free carbamazepine) were not significantly related to degree of clinical antidepressant or antimanic response.

However, CSF levels of carbamazepine-10,11-epoxide were significantly correlated with the degree of clinical response ($r = .67$, $p < .005$). Similar relationships were also observed in plasma where the epoxide, but not carbamazepine itself, was correlated with degree of clinical response. These data suggest that in these patients treated with an average of 1000 mg/day of carbamazepine, achieving plasma levels between 6 and 12 $\mu\text{g/ml}$, there is not a tight relationship between carbamazepine levels and clinical response. Similar observations have been made in the neurological literature in relationship to anticonvulsant efficacy. However, our data suggests the possibility that the -10,11-epoxide metabolite, which is reported to have anticonvulsant effects in animals, may also possess active psychotropic properties in man.

f. Comparison of Clinical Efficacy of Carbamazepine With That of Other Anticonvulsant Compounds. Clinical trials have been initiated to examine the relative efficacy of carbamazepine in comparison to other anticonvulsants such as phenytoin and valproic acid. In the first patient to complete a double-blind cross-over design, no evidence of clinical improvement was observed with these agents, while the patient was an unequivocal carbamazepine responder. These data suggest the possibility that biochemical or physiological properties peculiar to carbamazepine may, at least in this patient, be important to its psychotropic properties rather than relating to generalized anticonvulsant effects. Emrich and associates in Europe have, however, reported the successful use of valproic acid in a small number of lithium-resistant manic-depressive patients. Further clinical trials of these agents are indicated.

Although carbamazepine is a highly effective anticonvulsant, it is also useful in the treatment of a variety of paroxysmal pain syndromes which clearly do not involve an ictal process. Thus, the efficacy of carbamazepine clearly does not imply that subclinical seizures are the underlying pathophysiological mechanism in patients with affective illness. However, the properties mediating carbamazepine's anticonvulsant effects may nonetheless be related to its psychotropic properties. The clinical utility of the anticonvulsant carbamazepine raises the paradox of why electroconvulsive therapy is among the most effective treatments for acute manic and depressive illness. As detailed below, we have

documented that electroconvulsive seizures in the rat are paradoxically anticonvulsant to amygdala-kindled seizures. These data raise the possibility that common biochemical and physiological mechanisms of electroconvulsive therapy and the anticonvulsant carbamazepine could be related to their profile of therapeutic efficacy in both phases of affective illness.

g. Prophylactic Efficacy of Carbamazepine in Recurrent Affective Illness. In addition to carbamazepine's acute effects in manic and depressive episodes, our evidence suggests that carbamazepine may also be useful in the long-term prophylactic treatment of recurrent affective illness. In a series of seven patients followed for up to 51 months on carbamazepine, we have noted clear evidence of improvement during carbamazepine treatment compared to the year prior to treatment. These patients experience an average of 16.4 ± 5.7 total episodes of either mania or depression in the year prior to carbamazepine treatment, while this was reduced approximately 65% to 5.7 ± 2.4 episodes per year during carbamazepine prophylaxis. In addition, six of the seven patients were observed to relapse after brief periods of discontinuation of carbamazepine, further supporting the idea that active treatment with this agent was responsible for the improvement rather than a spontaneous frequency of cycling. All of these patients were previously non-responsive to lithium carbonate. Further clinical trials are indicated to explore the use carbamazepine alone, as well as in combination with lithium carbonate, in the long-term management of patients with recurrent affective and schizoaffective illness.

h. Clinical and Biological Predictors of Response to Carbamazepine. Patients with typical manic-depressive symptoms, as well as some atypical patients (with schizoaffective or psychotic or confusional elements), appear to respond to carbamazepine. It is of interest that a group of patients with rapid cycling affective illness who were not responsive to lithium carbonate responded to carbamazepine. Patients with more severe depression appear to respond better than those with less severe depression, as has been reported with other antidepressant compounds. Preliminary examination of EEG findings and the degree to which patients experience psychosensory phenomena, which are usually associated with epileptic-like processes, indicate that neither of these parameters appear to be predictive of carbamazepine response. Biological predictors of clinical response are also being studied. Preliminary evidence suggests that those with higher baseline CSF opiate binding activity show better antidepressant response to carbamazepine, while those with lower CSF cyclic-GMP are the better responders. Cerebrospinal fluid gamma-aminobutyric acid (GABA) and somatostatin levels are not predictive of clinical response. Patients with the lowest initial baseline medication-free serum sodium showed the greatest degree of hyponatremia on carbamazepine.

1. Studies of Carbamazepine's Mechanism of Action

1) Effects on Norepinephrine, Serotonin, Dopamine and GABA. Evidence in laboratory animals (Purdy et al.) suggests that carbamazepine blocks the reuptake of norepinephrine (NE) but also inhibits stimulated-induced release. We have observed, in collaboration with Dr. D. C. Jimerson and E. Gordon, that carbamazepine treatment significantly reduces the NE metabolite 3-methoxy-4-hydroxyphenylethylene-glycol (MHPG) in CSF of patients with affective illness. Cerebrospinal fluid NE itself, measured in collaboration with Dr. C. R. Lake, is

not significantly altered in the depressed patients, while the elevated levels of CSF NE in mania are decreased by carbamazepine. Noradrenergic effects of carbamazepine have indirectly been linked to its anticonvulsant properties.

The dopaminergic effects of carbamazepine are of considerable interest, but presently remain to be further clarified. There is substantial indirect evidence that carbamazepine does not act as a classical neuroleptic. It does not appear to block cocaine- or amphetamine-induced hyperactivity or stereotypy and does not raise HVA levels in rat brain or in the spinal fluid of our patients with affective illness, as do the classical neuroleptic treatments. Moreover, it has not been associated with the development of parkinsonian side effects or with the syndrome of tardive dyskinesia as have the neuroleptic drugs. Interestingly, carbamazepine produces slight, but statistically significant increases in serum prolactin in contrast to the major increases in prolactin achieved by traditional antipsychotic agents. These data suggest that carbamazepine may have important and differential effects on dopaminergic mechanisms that could be of potential importance in relation to its effects in either epilepsy or affective illness.

Alterations in GABA have been postulated both in affective illness (see below) as well as in the seizure disorders. Carbamazepine has been reported to decrease the turnover of GABA in animal studies, although brain levels are not altered by the drug. This is consistent with our data indicating that CSF GABA levels are not significantly decreased during treatment with carbamazepine compared to baseline levels. Cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA) is also not significantly affected by carbamazepine.

Preliminary data, in collaboration with Drs. M. Del Zompo and J. Tallman, suggest that carbamazepine may be interacting with the diazepam receptor as it significantly decreases diazepam receptor binding but not affinity. Cyclic nucleotides (cyclic-AMP and cyclic-GMP) have been postulated to play an important role in the therapeutic effects of a variety of psychotropic and anticonvulsant agents. While carbamazepine did not affect basal levels of these in the CSF of our affectively ill patients, probenecid-induced accumulations tended to be significantly reduced.

2) Effects of Carbamazepine on Endocrine and Peptide Systems. The possible effects of carbamazepine on endogenous opiate systems are of interest in relation to its efficacy in pain syndromes and the fact that it potentiates opiate-induced running activity in mice. There was no significant effect of carbamazepine on CSF opiate binding activity in 17 affectively ill patients, studied in collaboration with Drs. D. Naber, D. Pickar, and associates. Discrete effects of carbamazepine on regional opioid subsystems in brain remain to be ruled out, however.

Somatostatin was measured in CSF in collaboration with Drs. D. R. Rubinow, P. W. Gold, and S. Reichlin. Significantly lower levels of carbamazepine were found in depressed patients compared to normal volunteers or euthymic patients. It is of interest in this regard that carbamazepine significantly decreased CSF somatostatin, representing one of the first reports of a psychotropic drug affecting central nervous system (CNS) peptides in man.

The effects of carbamazepine on cortisol are noteworthy from several perspectives. Rubirow and associates have confirmed findings that carbamazepine induces escape from dexamethasone suppression, even in depressed patients who are showing clinical improvement. We have also observed that carbamazepine increases 24-hour excretion of urinary free cortisol in patients with normal baseline levels of cortisol. These data are consistent with observations by others that carbamazepine increases urinary free cortisol excretion in normal volunteers. Carbamazepine thus may be affecting regulation of the pituitary adrenal axis directly or through its effects on higher neural substrates in the limbic system or elsewhere. It is unlikely that the effects of carbamazepine on dexamethasone metabolism entirely account for escape from dexamethasone suppression, as urinary free cortisol was also increased. If further studies confirm that carbamazepine disinhibits the pituitary adrenal axis, it would represent one of the first demonstrations of a dissociation of clinical improvement from normalization of cortisol hypersecretion in depression.

Carbamazepine's effects on vasopressin are noteworthy from both a clinical and theoretical perspective. In contrast to lithium carbonate which produces the diabetes insipidus syndrome, carbamazepine has been used to treat diabetes insipidus. It has unequivocal antidiuretic properties which are manifest by its effects in producing mild hyponatremia (Dr. T. W. Uhde) and rare instances of water intoxication. During carbamazepine treatment decreased endogenous vasopressin is secreted in response to a hypertonic saline load, also consistent with an agonist role in this system (P. W. Gold and J. C. Ballenger). These findings are opposite those observed during lithium carbonate treatment. Dr. W. H. Berrettini has documented that carbamazepine is the one psychotropic drug tested to date that displaces ^{125}I -arginine-vasopressin binding from platelets, further suggesting that carbamazepine may have direct effects at the vasopressin receptor. The relationship of carbamazepine's antidiuretic effects to possible alterations in mood and cognition remain to be explored. Continued study of carbamazepine's biochemical effects, either alone or in comparison and contrast to lithium carbonate, may ultimately prove useful not only in further understanding its mechanism of action in affective illness, but also in helping to understand substrates underlying the affective disorders.

2. Approaches to Receptor Dysfunction in Affective Illness

a. Noradrenergic Receptors. Alpha-adrenergic receptors have been measured on platelets of drug-free patients with affective disorders and normal control subjects in collaboration with Dr. M. Kafka. The number of receptors measured by tritiated dihydroergocryptine was significantly increased in patients, while noradrenergic inhibition of prostaglandin E-1 stimulated cyclic-AMP production was not significantly altered. In contrast to these measurements in platelets, endocrine and behavioral responses have been studied following the acute intravenous administration of the alpha-2 receptor agonist clonidine, in collaboration with Drs. T. W. Uhde, L. J. Siever, and D. L. Murphy. Depressed patients showed significantly blunted growth hormone response to clonidine, which is thought to be an indirect marker of adrenergic receptor function. Consistent with its effects on decreasing firing of the noradrenergic locus coeruleus in animals, clonidine acutely decreased plasma NE and MHPG, measured in collaboration with Drs. C. R. Lake and D. C. Jimerson. Clonidine was associated with antianxiety effects measured on the Spielberger Rating Scale

in depressed and anxious patients. No significant effects on anxiety were observed following placebo administration or in the normal volunteer subjects. Clonidine's effects are consistent with the observations that CSF NE may be slightly elevated in depressed patients and in particular those with greater anxiety, compared to normal volunteers. However, CSF NE is markedly increased in manic patients compared to either of the other patient or control populations.

b. Dopaminergic Receptor Mechanisms. Interest in possible dopaminergic receptor alterations in affective illness and in response to pharmacological treatment strategies has recently been renewed. Data from other laboratories has indicated that from both a biochemical and electrophysiological basis, there is evidence that a variety of antidepressant treatment modalities including tricyclics, monoamine oxidase inhibitors, and electroconvulsive therapy may desensitize presynaptic inhibitory autoreceptors. This could result in increased dopaminergic neurotransmission and is consistent with our observations that some treatment-resistant depressed patients respond to the dopamine agonist piribedil. Others have confirmed these findings, indicating that another dopamine agonist, bromocriptine, may also be associated with antidepressant effects. We observed that the patients with lowest levels of homovanillic acid (HVA) in CSF responded best to the dopamine agonist piribedil, and also those with the lowest baseline HVA in CSF responded best to one night's sleep deprivation.

Diurnal variation in dopaminergic receptor responsivity has been suggested by the observations of greater hyperthermic response to apomorphine at 9:00 p.m. compared to that at 9:00 a.m. This index may provide a measure of dopaminergic responsivity at the level of the hypothalamus, while side effects such as nausea may reflect dopaminergic receptor function at the area postrema. Sedative and mood effects of apomorphine were significantly decreased in depressed patients compared to controls, however.

Studies in collaboration with Drs. D. C. Jimerson, N. R. Cutler, G. Brown, and P. W. Gold have suggested that depressed patients may have increased responsivity to the prolactin suppressing effects of apomorphine. Baseline prolactin levels tended to be lower and apomorphine-induced suppression of prolactin was significantly lower in 14 male depressed patients compared to male normal volunteers. These findings, suggestive of increased responsivity to a dopaminergic challenge, are of interest in relation to our observations of increases in tardive dyskinesia associated with depressed compared to manic phases of the illness in rapidly cycling patients with affective illness. Since tardive dyskinesia has been postulated to be related to dopaminergic receptor supersensitivity, increases in dyskinesia during depression and improvement during mania would be consistent with the endocrine observations.

c. Vasopressin Receptor Function. A vasopressin binding site on human platelets has been tentatively identified in studies in collaboration with Dr. W. H. Berrettini. Based on the potency of various compounds tested, preliminary data suggests that this binding site may have characteristics similar to that of the renal vasopressin receptor. These studies raise the possibility that one may be able to indirectly measure vasopressin receptor function in man, in addition to other measures such as that of vasopressin itself in CSF.

d. Glucocorticoid Receptors on Human Lymphocytes (studied in collaboration with Dr. D. C. Jimerson). Glucocorticoid binding to human lymphocytes is being assessed in medication-free patients and those on carbamazepine in an effort to further dissect the locus of cortisol dysregulation in a subgroup of patients with affective illness and to assess the possible mechanisms of carbamazepine in disinhibiting the pituitary adrenal axis and inducing escape from dexamethasone suppression.

3. Neurotransmitter Alterations in Manic and Depressive Illness

a. GABA. In collaboration with Dr. W. H. Berrettini, we have recently reviewed the literature on possible alterations in GABA-ergic mechanisms in affective illness. Indirect pharmacological data support a possible role of GABA in affective illness. Moreover, direct measurements of GABA in plasma and CSF provide some evidence of disturbed GABA function. Both plasma and CSF GABA are significantly lower in euthymic medication-free patients compared to normal controls. Three or four studies in the literature have reported low CSF GABA in depression compared to control groups. We have observed significantly lower levels in individuals studied longitudinally during depressed compared to manic phases of the illness. Dr. Berrettini, in conjunction with Dr. E. Gershon, has collected further evidence that GABA may in part be regulated at a genetic level, as well as fluctuating in a state-related fashion. Plasma GABA levels were significantly correlated in identical twin pairs. Dr. Berrettini has also measured GABA transaminase (GABA-T) and found this enzyme to be significantly lower in affectively ill patients compared to normal volunteers. These studies, suggesting possible GABA alterations in affective illness, are of interest in relation to recent reports that GABA agonists may have antidepressant effects, and that several agents reported to be effective in the treatment of recurrent affective illness (electroconvulsive therapy, carbamazepine, and valproic acid) all decrease GABA turnover. Dr. Berrettini has also observed that patients treated with lithium carbonate have significantly higher plasma and CSF GABA levels compared to untreated individuals. These data are also consistent with several reports that lithium carbonate may influence GABA-ergic mechanisms.

b) Noradrenergic Function in Affective Illness. In addition to the studies reported above of alterations in CSF NE in anxious depressed patients and in manic patients, we have observed significant state-related alterations in CSF NE in rapidly cycling manic-depressive patients. Similar alterations have been documented in plasma MHPG measured by Dr. D. C. Jimerson, with higher levels in manic compared to depressed phases of the illness in patients studied longitudinally. In normal volunteers plasma MHPG was observed to correlate significantly and negatively with severity of depression, hypochondriasis, and psychasthenia scales measured on the Minnesota Multiphasic Personality Inventory (MMPI). These data raise the possibility that noradrenergic mechanisms may be associated with the normal as well as pathological range of affective function.

Measurements of noradrenergic function in blood, urine, and spinal fluid of these affectively ill patients, in collaboration with Drs. D. C. Jimerson and J. C. Ballenger, are also helpful in clarifying the role of interrelationships between noradrenergic measures in different body fluids.

In addition to the state-related alterations in noradrenergic function, we have been interested in assessing the relationship of this system to the longitudinal course of affective illness, as assessed by life chart methodology. We have observed that those patients with higher CSF NE had greater numbers of episodes in the year prior to NIMH admission ($r = .61, p < .05$). In addition, those with higher CSF NE during the depressive state experienced greater numbers of weeks ill in the year prior to NIMH admission ($r = .76, p < .001$). We have also followed a group of patients to assess social functioning an average of 3.5 years following discharge from NIMH (unpublished data with Dr. R. J. Savard). Patients who had poor social functioning measured in the social and leisure activity subscale of the Social Adjustment Scale had higher CSF VMA ($r = .66, p < .02, n = 13$) and higher CSF NE ($r = .80, p < .005, n = 11$). These findings, taken together, suggest that increases in noradrenergic function measured during an acute episode of depression may be positively related to the longitudinal course of affective illness and to greater frequency of cycling as well as poorer prognosis variables. These are among the first observations of biological correlates associated with the longitudinal, rather than acute state-related, course of affective illness.

4. Peptides in CSF: Interrelationships with Neurotransmitter and Behavioral Alterations

a. Introduction. More than 20 neuropeptide substances have been suggested as putative CNS neurotransmitters or modulators. We have recently reviewed the literature indicating that essentially all of these substances have been tentatively identified and measured in the CSF of man. This provides one strategy for attempting to identify peptidergic alterations in neuropsychiatric disorders and to examine their postulated relationship to alterations in behavior, cognition, and affect. Neuropeptides have recently been reported to co-exist in the same neurons with classical neurotransmitter substances. Again, the CSF provides an opportunity for studying the potential interaction between both classical neurotransmitters and the recently discovered neuropeptides.

b. CSF Opiate-like Substances. In collaboration with Drs. D. Pickar, J. C. Ballenger, D. Naber, D. R. Rubinow, W. E. Bunney, Jr., and F. K. Goodwin, we have measured opiate substances in CSF utilizing a measure of both total CSF opiate binding activity and immunoreactive beta-endorphin. Total CSF opiate binding activity was not significantly different in depressed, manic, or improved patients compared to normal volunteers. Cerebrospinal fluid opiate binding activity from baseline and probenecid lumbar punctures was correlated ($r = .73, p < .01$) in 16 patients, indicating the relative stability of this measure across two different lumbar puncture procedures. Although there were no significant differences related to affective state, interesting relationships between CSF opiate activity and anxiety were observed. In depressed patients, those with higher nurse-rated anxiety showed significantly higher opiate binding activity ($r = .47, p < .01, n = 36$). These data are of particular interest in relation to the recent observation that a variety of stresses may be associated with the release not only of ACTH but also beta-endorphin in several experimental paradigms. The relationship between opiate activity in CSF and anxiety is also intriguing in relation to the differential findings in normal volunteers. Utilizing a different measure of anxiety, i.e. self-rated state anxiety at the time of the lumbar puncture, it was observed that normal volunteers with higher

CSF opiate binding activity showed significantly lower levels of subjective self-rated anxiety ($r = -.40$, $p < .05$, $n = 37$). These findings suggest that there may be complex interrelationships between opiate substances in CSF and different measures of acute and chronic anxiety in normal volunteers and depressed patients.

Cerebrospinal fluid beta-endorphin measured by radioimmunoassay was also not significantly different in unipolar and bipolar depressed patients compared to manic patients or normal volunteers. Preliminary evidence suggested that CSF immunoreactive beta-endorphin was differentially related to personality characteristics in female compared to male volunteers. Male volunteers showed a positive relationship between beta-endorphin and assaultiveness on the Buss-Durkee Rating Scale ($r = .77$, $p < .0002$) and on the Trait Hostility Scale ($r = .48$, $p < .04$), while female volunteers showed positive correlations between immunoreactive beta-endorphin and depression, social introversion, and negativity. These highly preliminary findings require replication, but are suggestive of the possibility that peptides measured in CSF might be associated with alterations in anxiety and certain personality variables, even though they are not different in diagnostic subgroups of patients with affective illness.

c. Somatostatin in CSF. Cerebrospinal fluid somatostatin has been measured in CSF of affectively ill patients and normal volunteers by sensitive radioimmunoassay in collaboration with Drs. S. Reichlin, D. R. Rubinow, and P. W. Gold. Dr. Rubinow found that CSF somatostatin was significantly decreased in depressed patients compared to those restudied in the euthymic state or compared to normal volunteer controls. These findings replicate those of Gerner et al. of state-related decreases in CSF somatostatin in depression. Cerebrospinal fluid somatostatin in affectively ill patients was significantly and inversely correlated with number of hours of sleep in the night prior to the lumbar puncture. These data are consistent with those in the animal literature that somatostatin decreases a variety of sleep parameters including total sleep. Somatostatin has recently been reported to be co-stored in noradrenergic neurons. It was thus of particular interest to observe interrelationships between somatostatin and noradrenergic measures. Cerebrospinal fluid somatostatin was inversely correlated with CSF NE ($r = -.52$, $p < .02$). This represents one of the first reports of the exploration of the interaction of classical and putative neuropeptide transmitters in man. It is of particular interest not only to the issue of co-storage, but the regulation of somatostatin by NE. As noted above, carbamazepine significantly decreased CSF somatostatin, while other psychotropic drugs produced no significant alterations and the relatively specific blocker of serotonin reuptake, zimelidine, significantly increased CSF somatostatin. These findings thus open new areas for exploration of the possible role of somatostatin decreases in depression, relative increases in relationship to degree of sleep disturbance, and in the possible mechanism of action of carbamazepine which has an interesting spectrum of clinical efficacy in affective illness, seizure disorders, and paroxysmal pain syndromes.

d. ACTH and Its Peripheral Target Hormone Cortisol. Drs. D. R. Rubinow, P. W. Gold, and J. C. Ballenger have extensively studied pituitary adrenal dysregulation in affective illness. They have observed significantly higher excretion of urinary free cortisol in unipolar and bipolar depression compared to normal volunteers with significantly lower levels in manic patients.

These findings are paralleled by a large literature of well-documented and replicated studies indicating that approximately 50% of depressed patients show evidence of cortisol hypersecretion measured either by escape from dexamethasone suppression, increased urinary free cortisol, or altered diurnal variation of cortisol secretion. In addition, Dr. Rubinow has documented marked state-related alterations in urinary free cortisol secretion and highly significant correlations in 8:00 a.m. plasma cortisols with severity of depression in cycling manic-depressive patients studied longitudinally. It is of interest in relationship to the evidence of co-secretion of ACTH and beta-endorphin that, within the depressed patient population, those with higher urinary free cortisol secretion showed higher levels of CSF opiate binding activity. In our studies of urinary free cortisol and in the literature on dexamethasone suppression, severity of depression has not been well correlated with evidence of pituitary adrenal axis disinhibition. It was particularly noteworthy to find that patients with higher levels of urinary free cortisol showed greater cognitive impairment on the Halstead Categories test of abstracting ability ($r = .48, p < .01$). These findings suggest that patients with higher levels of urinary free cortisol are more cognitively impaired, which is of interest in relationship to the high density of glucocorticoid binding sites measured in limbic structures such as the hippocampus which are thought to be critically involved in some aspects of learning and memory function. It is possible that either the high levels of cortisol itself or the neurochemical alterations underlying this abnormality are associated with this objective measure of cognitive impairment. These data are of some theoretical relevance, as well as of possible clinical significance since depressed patients often have marked complaints of subjective decreases in cognitive and memory capacity.

d. Vasopressin and Oxytocin. Vasopressin and oxytocin are of considerable interest since a large body of experimental data in animals and preliminary studies in man suggest that they may have effects on learning and memory. Vasopressin has been measured in plasma and CSF in collaboration with Drs. P. W. Gold, D. R. Rubinow, and G. Robertson. Dr. Gold has observed that CSF values in non-psychotic bipolar depressed patients were significantly lower than those in the manic phase of the illness. In contrast, CSF oxytocin, measured in collaboration with Dr. D. Fisher, was found by Dr. Gold to be significantly decreased in manic patients compared to normal volunteers. The possible relationships of these findings to the syndromal and symptomatic alterations in mania and depression remain to be further explored but appear to be of considerable interest in their own right, as well as in relation to their serving as possible markers of hypothalamic dysfunction. Preliminary evidence suggests that vasopressin may be secreted directly into CSF independently of alterations in its peripheral levels. These and related data suggest that study of peptides in CSF may provide useful indirect markers of CNS peptide function and provide a basis for studying alterations in relationship to a variety of neuropsychiatric symptoms and syndromes. Drs. Rubinow, Gold, and Weingartner are studying the effects of infused oxytocin and vasopressin on mood and cognitive capacities in affectively ill patients and normal volunteers. Initial data suggest that vasopressin enhances, while oxytocin impairs, certain aspects of cognition.

5. Life Charting the Course of Affective Illness

We have recently completed the first phase of analysis of the life course of illness in 66 unipolar and bipolar patients. In addition to this detailed retrospective life chart evaluation, cyclicality within NIMH has been precisely characterized. Differential characteristics of unipolar patients have been noted. Compared to bipolars, unipolar patients had significantly greater numbers of weeks hospitalized per years ill, but decreased number of total depressive episodes or episodes in the year prior to NIMH admission, while they experienced more weeks ill in the year prior to NIMH admission. Thus, their illness was characterized by decreased cyclicality but equal or greater impairment in functioning. These differences persisted during hospitalization at NIMH, with unipolar patients showing decreased number of depressive episodes compared to bipolar patients.

Female compared to male patients showed an increased proclivity to rapid cycling and significantly greater number of episodes in the year prior to NIMH admission (5.9 ± 1.1 , $n = 34$ compared to 1.7 ± 0.2 , $n = 29$). Females were overly represented in the group of rapid cycling patients. Male and female rapid cycling patients ($n = 20$) compared to slow cycling patients ($n = 29$) had significantly longer duration(s) of illness, more hospitalizations for depression, greater numbers of weeks hospitalized, as well as greatly increased total lifetime episodes of affective illness (56.7 ± 18.6 compared to 8.2 ± 1.2). The rapidity of cycling, defined as four or more episodes in the year prior to NIMH hospitalization, continued to be an excellent predictor of the course of illness at NIMH with rapid cyclers showing significantly more manic, depressive, and total episodes at NIMH. In the total group of 47 bipolar patients the number of episodes in the year prior to NIMH admission was highly correlated with the number of episodes observed during NIMH hospitalization ($r = .69$, $p < .0001$). Thus, the prior course of cycling appears to be the best predictor of subsequent course of illness. Numbers of episodes of mania and numbers of episodes of depression prior to NIMH hospitalization were highly correlated within 32 patients studied ($r = .90$, $p < .0001$). This same symmetry between number of observed manic and depressive episodes was again documented during the NIMH hospitalization where number of manic episodes correlated with number of depressive episodes ($r = .91$, $p < .001$) in 49 bipolar patients studied.

A substantial number of depressed patients were observed to show a progressive increase in rapidity of cycling as a function of episode number as observed earlier by Kraepelin and more recently by Goodwin, Zis, Grof, and associates. Thus, the study of the longitudinal course of affective illness provides a template not only for assessing the phenomenology of the illness and its response to treatment interventions with agents such as lithium and carbamazepine, but also refocuses on possible biological mechanisms underlying the recurrent and, at times, progressive aspects of affective illness. For example, we have noted above findings of increased noradrenergic function in depression associated with rapidity of cycling. Studies in laboratory animals of behavioral sensitization to stimulants and stressors, and of electrophysiological kindling, may provide insights into different types of mechanisms underlying the progressive evolution of behavioral disturbances in response to repetition of the same stimulation over time.

We suggest that the life charting process is a useful clinical as well as research tool and may help focus on possible environmental precipitants and dynamically significant events and stresses that may be temporally related to affective episodes. It also allows precise characterization of the degree of longitudinal response to newly available pharmacological agents. Recent data of Wehr and Goodwin have emphasized that some pharmacological interventions such as the tricyclic antidepressants may actually result in increased rapidity of cycling. The life chart methodology provides a useful instrument for following this problematic side effect. We have also observed in several patients that lithium carbonate may paradoxically increase the rate of rapid cycling in addition to significantly decreasing the duration of recurrent depressive episodes. The particular vulnerability of female patients to experience extremely rapidly cycling manic-depressive illness would appear a fruitful area of further study. It also helps focus on possible endocrine concomitants of this process.

6. Menstrually-Related Mood Dysfunction

A relationship between mood and behavior and menstrual function has been described with respect to a number of disorders including premenstrual tension, post-partum depression, epilepsy (so-called catamenial epilepsy), anorexia nervosa, pseudocyesis, secondary amenorrhea, and menopausal dysphoria. Dr. D. R. Rubinow has initiated a series of studies to investigate the relationship between mood disorders and the menstrual cycle. These studies include: development of a questionnaire which is being employed to help determine the incidence and nature of affective symptoms in relation to the menstrual cycle; assessment of the precision of the relationship between mood changes and the menstrual cycle utilizing daily self ratings and daily temperature recordings; investigation of hormonal activity employing periodic blood samples and neuroendocrine tests; and assessment of the efficacy of progesterone, a synthetic progestin, and carbamazepine in the treatment of established menstrually-related mood syndromes. The results of such a study may: 1) determine whether a specific association between depressive symptoms and menstrually-related phenomena (menstruation, post-partum depression, menopause, hormone-induced behavioral change) can be established; 2) reveal the incidence of the entrainment of depressive symptoms to the menstrual cycle; 3) help elucidate the nature of the "switch" mechanism in affective disorders and periodic psychosis; and 4) determine the efficacy of pharmacologic agents believed useful in the treatment of menstrually-related mood disorders.

At this point, questionnaires have been filled out by 75 women, and eight women are entering the hormonal assessment phase of the study, having completed the baseline evaluation phase.

7. Depressive Subtypes and Symptoms in Relation to Regional Localization of Function

a. Atypicality of Depression. In collaboration with Dr. E. K. Silberman, we have devised an atypicality of depression rating scale in order to more precisely characterize the range of atypical depressive presentations in patients who otherwise meet formal Research Diagnostic Criteria for primary affective illness. Older patients and those with bipolar I affective illness had more typical presentations than those of unipolar or bipolar II patients.

The more typical patients showed increased rapid cycling in the year prior to NIMH admission although, interestingly, decreased numbers of total hospitalizations compared to the atypical patients. Atypical depressed patients also showed more variance in biological measures such as those of the noradrenergic system, further suggesting that the range of clinical presentations may be related to the range of biological variables that have been hypothetically linked to depressive illness. It was of interest that both typical and atypical depressed patients showed similar degrees of cortisol hypersecretion.

b. Psychosensory Phenomena. In collaboration with Dr. E. K. Silberman, we have developed an interview rating scale designed to measure signs and symptoms that are usually associated with psychomotor epilepsy (complex partial seizures). We have studied these phenomena in patients with primary affective illness without evidence of seizure disorders, in patients with documented evidence of temporal lobe epilepsy, and in a medical control group of hypertensive patients. Compared to the control group, patients with both affective illness and epilepsy showed a markedly increased incidence in number of these signs and symptoms. To the extent that psychosensory distortions and related symptoms usually associated with temporal lobe epilepsy are occurring with a high incidence in patients with primary affective illness, it might suggest that these classically affectively ill patients have impairment in at least partially overlapping neural substrates to those involved in psychomotor epilepsy. We are currently exploring whether those patients with greater numbers of psychosensory symptoms respond better to the anticonvulsant carbamazepine, although preliminary data suggest that this is not the case.

c. Procaine Infusions in Patients with Affective Illness and Controls. Several types of studies suggest that procaine and related local anesthetics may show some relative selectivity in their effects on limbic system substrates. We are examining behavioral, physiological, and electroencephalographic responses to dose-related increases in intravenous procaine administration in order to assess whether there are altered thresholds of effects in different diagnostic groups and to assess whether responses may predict subsequent response to the anticonvulsant carbamazepine. Infusions in our first two patients have been completed and reveal dose-related alterations in affect and sensory distortion, as well as EEG alterations in the 40 cycle/second band thought to reflect activity in limbic structures (data analyzed in collaboration with Drs. R. Adamec and K. Livingston). These infusion protocols, in collaboration with Drs. F. W. Putnam, C. H. Kellner, and S. Sato of the EEG laboratory, may provide one approach to assessment of limbic system function in man.

d. Psychological, Structural, Metabolic, and Electrophysiological Approaches to Regional Brain Function in Affective Illness. A variety of psychological test batteries are employed to assess possible alterations in regional brain function in patients with affective illness including the Luria Battery, the Halstead Categories Test, tachistoscopic presentation to assess hemispherical laterality, and other cognitive tests studied in collaboration with Dr. E. K. Silberman. Consistent with patients' subjective sense of cognitive impairment during depression, marked impairment in cognitive function has been documented on the Halstead Categories Test. Degree of cognitive dysfunction correlates with increases in urinary free cortisol secretion. The Luria Battery

provides another approach to assessment of possible regional areas of dysfunction and has been completed in 27 patients.

Computerized axial tomography (CAT) scans have been performed on our patients with affective illness and reveal a similar range of increased ventricular brain ratios comparable to those observed in schizophrenic patients. We are currently assessing the clinical and biological concomitants of this evidence of altered brain structure in a subgroup of affectively ill patients (in collaboration with Drs. C. H. Kellner and W. H. Berrettini).

As described in detail elsewhere, topographic mapping of EEG frequencies and averaged evoked response is being conducted in collaboration with Dr. M. S. Buchsbaum. These studies, in conjunction with positron emission tomography (PET scan), may provide important evidence of electrophysiological and/or metabolic regional dysfunction in affective illness. These findings can then be compared with ongoing psychological, longitudinal, physiological, and biochemical assessment of affectively ill patients in order to complete a coherent and comprehensive assessment of possible interrelationships of these measures in affective illness.

8. Laboratory Studies of Behavioral Sensitization and Electrophysiological Kindling

a. Stimulant-induced Behavioral Sensitization. A series of studies have been designed to investigate the mechanisms underlying increased behavioral responsivity to the same dose of a psychomotor stimulant such as cocaine. Animals administered cocaine (10 mg/kg, i.p.) once daily showed increasing amounts of locomotor hyperactivity and stereotypy to the same dose over time. An environmental context and conditioning component has been demonstrated. Animals repeatedly treated with cocaine in the context of the test cage showed greater degrees of hyperactivity and stereotypy than animals receiving identical doses in a different environment. Significant differences also existed when animals were challenged with a saline injection, again suggesting a conditional component to cocaine-induced behavioral sensitization.

Studies are now in progress in collaboration with Dr. K. Zander to assess cross sensitization between cocaine-induced hyperactivity and several types of stresses such as those induced by tail pinch. It appears that type of stress, its intensity, and longitudinal time course are important determinants of whether animals will show increased or decreased responsivity to a cocaine challenge. Some aspects of the response to repeated stress showed clear-cut sensitization effects, while others appeared to show adaptation or tolerance. For example, 40 kHz vocalization showed increasing amplitude of response to repetition of the same level of tail pinch stress over time.

The neuropeptide vasopressin has been implicated in the modulation of learning and memory in some animal models. It had also been reported to affect the development of tolerance to opiates and alcohol in some studies. Since behavioral sensitization to cocaine also involved long-term adaptation to repeated administration of the same pharmacological stimulus over time, we were interested in assessing whether it might have important effects in this paradigm. Brattleboro homozygote rats lacking vasopressin showed deficient onset,

maintenance, and persistence of cocaine-induced behavioral sensitization compared to their heterozygote litter-mate controls. Another group of Brattleboro homozygotes were able to show similar degrees of acute reactivity to high doses of cocaine indicating that the homozygotes were not just unable to show similar degrees of motor activation. These studies suggest that vasopressin or its secondary biochemical alterations might play a role in the altered rate of behavioral sensitization.

We have subsequently replicated the original findings of deficient behavioral sensitization in animals lacking vasopressin and have extended these observations with the finding that vasopressin replacement will reverse the deficit in cocaine-induced behavioral sensitization. Although secondary effects of vasopressin on other biochemical systems cannot be completely ruled out in these studies, the data suggest that vasopressin may influence behavioral adaptation in the direction of increased responsivity, such as observed in the cocaine model, just as it has been reported to alter the development of drug-induced tolerance.

In order to examine whether endogenous opiates which have been implicated in learning and memory paradigms and reinforcement behavior might be involved in the development of behavioral sensitization, the opiate antagonist naloxone was administered prior to the injections of cocaine. Naloxone administration decreased the progressive development of cocaine-induced horizontal motor activity, while it facilitated vertical rearing activity compared to controls. Further studies are required to clarify the nature of the apparently complex interaction between naloxone-reversible processes and the development of cocaine-induced behavioral sensitization.

Clear-cut effects of gender are observed in the behavioral sensitization paradigm. Female compared to male rats are more responsive to the same dose of cocaine. They demonstrate similar behavioral sensitization to repeated injections of cocaine at approximately half the dose (5 mg/kg) of that used in males (10 mg/kg, i.p.). At the 10 mg/kg dose females do not show adequate sensitization as they are already maximally hyperactive on day 1. These findings suggest the possibility that female sex hormones may be an important factor in the behavioral response to cocaine. These findings are of potential interest in relation to recent reports of the interaction of female sex hormones with dopaminergically mediated behaviors and alterations in dopamine receptor sensitivity. Altered response to psychomotor stimulants may also be of relevance to the observation that female compared to male patients show a higher incidence of unipolar affective disorder and are disproportionately represented in the group of extremely rapidly cycling manic-depressive patients.

b. Electrophysiological Kindling. Repeated, intermittent electrical stimulation of the brain results in increasing duration, spread, and complexity of electrical after-discharges culminating in the appearance of major motor seizures to a previously subthreshold stimulation. We have employed this procedure, as described by Goddard et al., in order to study long-lasting changes in neural and behavioral excitability that accompany this process. Following electrical kindling of the amygdala, rats showed decreased spontaneous and cocaine-induced exploratory activity, while they show increased convulsive susceptibility to a related local anesthetic lidocaine. There is increasing

evidence that lidocaine administration may in itself lead to a kindling-like sensitization process. Repeated injections of the same dose of lidocaine (65 mg/kg, i.p.) leads to an increasing incidence, severity, and duration of seizures to the same dose over time. This effect does not appear to be a pharmacokinetic one, as blood levels of lidocaine and its metabolite are not increased with chronic administration. Moreover, if lidocaine-induced excitability and seizures are blocked by the co-administration of diazepam, no seizure sensitization occurs. Finally, repeated lidocaine-induced seizures sensitize to electrophysiological kindling of the amygdala such that amygdala-kindling proceeds three times faster following lidocaine pretreatment compared to saline controls. These data suggest some degree of cross sensitization between electrical and chemical modes of kindling.

Behavioral alterations persist in the interictal period following lidocaine-induced seizures. In collaboration with Drs. L. Sokoloff, C. Kennedy, and their associates in the Laboratory of Neurochemistry, it has been demonstrated that lidocaine-induced seizures relatively selectively increase metabolic activity in limbic system structures, particularly amygdala, hippocampus, perirhinal, and cingulate cortical areas. It is, thus, of interest that increases in irritability and resistance to capture are prominent following lidocaine seizures but not following seizures induced by electroconvulsive shock or pentylenetetrazol (Metrazol). The changes in irritable and aggressive behavior following lidocaine seizures persist for some days into the interictal period. This paradigm would therefore appear to be a useful one in exploring the relationship of seizures with some specificity for limbic structures to alterations in aggressive behavior.

c. Electroconvulsive Shock Inhibits Amygdala Kindling. The clinical utility of an anticonvulsant such as carbamazepine appears paradoxical in relation to electroconvulsive therapy or the induction of major motor seizures also having therapeutic efficacy in both manic and depressed phases of affective illness. One possible explanation of this paradox emerges from two separate studies in collaboration with Dr. F. W. Putnam and N. Contel demonstrating that the major motor seizures of electroconvulsive shock (ECS) are themselves anticonvulsant to amygdala-kindled seizures. Pretreatment with ECS six hours prior to amygdala kindling markedly inhibits development of kindled seizures compared to sham ECS or compared to ECS administered immediately after kindling. In a second study, we used a more clinically relevant paradigm. Animals were kindled to their first stage for major motor seizure and then were treated with single or seven daily ECS or sham ECS. Following this seven-day interval, amygdala kindling was resumed. Chronic ECS, but not one ECS followed by a seven-day delay, markedly inhibited amygdala-kindled seizures for up to five days compared to sham ECS controls. Taken together these two studies indicate that the major motor seizures of ECS can, in two different time frames, exert marked anticonvulsant effects on amygdala-kindled seizures. These data raise the possibility that the efficacy of electroconvulsive therapy in patients with affective illness could be related to effects mediating its anticonvulsant actions.

Carbamazepine is a potent inhibitor of amygdala kindling. As noted above, we have recently observed that carbamazepine-10,11-epoxide is more highly correlated with the degree of psychotropic response in our patients than is the

parent compound. We have demonstrated that the metabolite carbamazepine-10,11-epoxide is also effective in inhibiting amygdala-kindled seizures, although it is slightly less potent than carbamazepine itself.

D. Proposed Course of Project

As carbamazepine is emerging as an effective treatment modality in some patients with manic-depressive and schizoaffective illness, we will attempt to further delineate clinical and biological markers of carbamazepine response. Preliminary evidence suggests that many patients who clearly do not respond to lithium carbonate will respond to carbamazepine. It will be increasingly important to establish whether response to carbamazepine compared to lithium carbonate delineates separate subgroups of patients with affective illness. It is also possible that carbamazepine may be more effective in later stages of the illness, particularly when the patients are in a treatment-resistant rapid cycling phase of illness. Genetic variables and patients with a family history of psychiatric illness will be examined in relationship to carbamazepine. Patients with mild EEG abnormalities, cognitive alterations, or alterations in psychosensory function will also be examined in relationship to anticonvulsant response. The degree of generalization of carbamazepine response to other anticonvulsant agents such as phenytoin or valproic acid will be another area of both clinical and theoretical import. This is also particularly the case in light of our recent findings that electroconvulsive therapy may be exerting potent anticonvulsant effects on limbic system seizures. Are anticonvulsant effects of a variety of treatment modalities linked to therapeutic response in affective illness? Carbamazepine is clearly useful in pain syndromes that do not involve a convulsive process, and effectiveness of anticonvulsant agents in a subgroup of patients with affective illness does not imply an underlying ictal process. The possible mechanisms of action of carbamazepine studied in our clinical population, as well as in behavioral pharmacological models and at more basic molecular levels, will also be pursued.

Topographic mapping of electroencephalographic activity and PET scan techniques will be explored in collaboration with Dr. M. S. Buchsbaum, not only in affectively ill patients compared to controls, but also as they might predict or correlate with treatment response. Further clinical and laboratory work will be pursued to investigate whether carbamazepine's anticonvulsant metabolite carbamazepine-10,11-epoxide has active psychotropic properties.

The interrelationship of classical neurotransmitter substances with the putative CNS neurotransmitter peptides will be explored in both patients with affective illness and anxiety disorders in collaboration with Drs. D. C. Jimerson and T. W. Uhde. A variety of techniques are in place for measurement of neurotransmitter and receptor function in both classical neurotransmitter systems and in the peptide systems in man. These will be correlated with behavioral alterations and changes in mood and cognitive functioning in patients with mood and anxiety disorders.

As described in detail in Project #Z01 MH 00071-02 BP, Dr. T. W. Uhde will continue to explore the similarities and differences in patients with panic anxiety syndromes and those with affective illness in terms of acute symptomatology, longitudinal course of illness, and response to pharmacological agents.

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Catecholamines appear to be altered in both the mood disorders and in panic anxiety disorders. Response to treatments which act on catecholamine systems such as clonidine will be compared and contrasted in both patient populations. The clinical utility of carbamazepine will also be explored in this syndrome.

Dr. F. W. Putnam will be completing his studies of psychological, psychophysiological, and neural mechanisms underlying patients with multiple personality syndrome. An extensive questionnaire has been constructed which will better delineate symptoms and course of illness as well as incidence of multiple personality syndrome.

Dr. D. R. Rubinow is also developing a new combined inpatient and outpatient focus on patients with menstrually-related exacerbation of mood and behavior disorders. He will be examining this problem from a clinical and endocrinological point of view, and as a model for studying the acute onset and offset of affective dysfunction. Similar studies will be pursued utilizing sleep deprivation which represents another non-pharmacological means of inducing rapid and non-pharmacologically related improvement in mood as well as examining mechanisms that may underlie exacerbation of depression which occurs regularly when patients return to sleep. Work in animal models will focus on examination of possible mechanisms underlying behavioral sensitization and electrophysiological kindling. In collaboration with P. Marangos and J. Patel, neurotransmitter receptors, protein phosphorylation, and ion channels will be examined as possible mediators or modulators of the electrophysiological kindling paradigm. Studies of behavioral and biochemical response to repeated stress will be performed in collaboration with Dr. A. Pert. The examination of cross sensitization between psychomotor stimulant changes and stress-induced behavioral alterations will be further explored in collaboration with Dr. K. Zander. The role of environmental context and conditioning will also be examined in these paradigms.

E. Significance to Biomedical Research and the Program of the Institute

Findings in several research areas are of considerable clinical and theoretical significance. Carbamazepine is emerging as a new treatment for manic-depressive illness; it is effective in some patients who do not respond to lithium carbonate. In addition, clinical and basic work exploring the mechanism of action of this compound alone or in comparison to lithium carbonate may provide new leads to the understanding of mechanisms of action of effective antimanic and antidepressant drugs and mechanisms underlying affective dysregulation. Study of endocrine and peptide substances in man and animals may also provide new conceptual and practical approaches to the relationship between manic and depressive symptoms and biochemistry. Examination of the interaction between classical neurotransmitters and the peptides should prove fruitful in understanding normal and pathological functioning. The multidisciplinary assessment of our patients' mood, behavior, cognition, physiology, and biochemistry will allow more precise characterization of important biobehavioral relationships.

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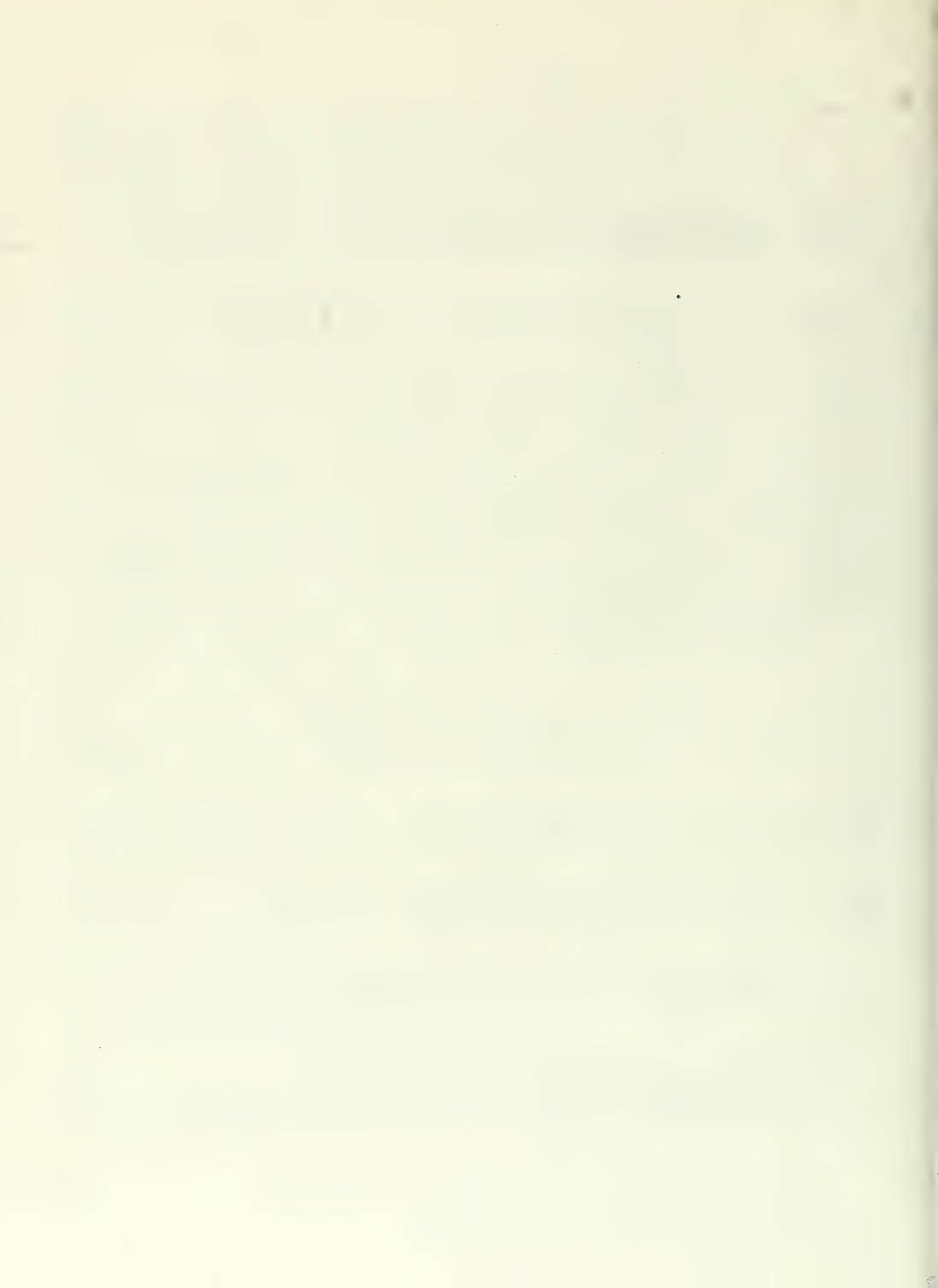
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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00071-02 BP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Patients</u> with pathological degrees of <u>anxiety</u> who meet DSM III criteria for generalized anxiety, panic or phobic disorders are evaluated using psychological, physiological, and biochemical methodologies. Particular attention is given to the role of the <u>noradrenergic neurotransmitter system</u> as assessed by: 1) measurement of the metabolite MHPG in urine, plasma, and CSF; 2) adrenergic receptor number and function in platelets; and 3) neuroendocrine and behavioral response to the alpha-2 adrenergic agonist <u>clonidine</u>. Research investigating the relationship of noradrenergic function to other neurotransmitter systems such as those which influence opiate, serotonin, and GABA-benzodiazepine function also have been initiated. Other approaches to understanding the pathophysiology of anxiety and its potential treatment with clonidine and <u>carbamazepine</u> will be explored. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

I. Project Description

A. Objectives

This project employs a multidisciplinary team in the study and treatment of pathological anxiety and related mood disorders.

B. Methods Employed

1. Subjects

a. Patients who meet Research Diagnostic Criteria for panic, phobic, and generalized anxiety disorders are candidates for participation in the project. Patients are studied and treated on the 3-West Clinical Research Unit, Section on Psychobiology or through the Section's Outpatient Division. A number of previously validated scales to measure state and trait anxiety are utilized and an analogue anxiety scale has been developed to more adequately assess the relationship among state anxiety, phobic anxiety, and avoidance behavior.

b. Normal volunteers are also accepted into the project to provide control data as well as to assess the relationship between normal state anxiety and selected psychological and biological variables.

2. Psychological and Biological Evaluation

a. Baseline Evaluation. During an initial evaluative period patients undergo extensive neurological, psychological, biochemical, and neuro-physiological evaluation. This initial evaluation is indicated due to the heterogeneous nature of the panic and phobic disorders. Anecdotal reports suggest that many medical illnesses may present as or exacerbate pre-existing conditions of pathological anxiety. However, no research has systematically studied a large number of panic and phobic patients to determine the incidence and prevalence of these associated disorders.

b. Life Chart Methodology. A modified life chart technique has been developed to plot the frequency, intensity, and interval between panic attacks so that the development, recurrence, and progression of the panic and phobic disorders can be assessed. This is an important aspect of the project because few systematic studies have been conducted on the natural progression of these disorders.

c. Caffeine and Anxiety. In collaboration with Dr. J.-P. Boulenger, a caffeine inventory has been developed to assess the effects of caffeine on anxiety and related symptoms in panic anxious patients and normal volunteers.

d. Physiology. Motor activity is measured continuously every 15 minutes with a miniaturized activity monitor developed by Dr. T. Colburn. A number of investigators have demonstrated a relationship between state anxiety and noradrenergic-related substances (plasma MHPG, urinary MHPG, CSF MHPG) but the effects of motor activity on these measurements remain controversial. The

computerized activity monitor provides a unique method by which the relationship among these variables may be effectively investigated.

e. Sleep Research. Electroencephalographic sleep recordings are obtained for three consecutive nights. Although many panic anxious patients, like endogenously depressed individuals, have improved sleep following treatment with tricyclic and monoamine oxidase inhibitors, nothing is known about the sleep architecture of panic and phobic anxious patients. In collaboration with Dr. J.C. Gillin, this ongoing research represents the first attempt to our knowledge to evaluate the sleep profile of this patient population.

f. Insensitivity Index. Using threshold and signal detection methodology designed by Dr. M.S. Buchsbaum, an index of pain insensitivity is obtained in patients and normal volunteers following the intravenous administration of clonidine 2 µg/kg and placebo.

g. Galvanic Skin Response. The effects of clonidine, carbamazepine, and selected standard anxiolytics on physiological measures of galvanic skin response, reaction time to auditory tones, pulse, and respiratory rate are studied in panic and phobic anxious patients and age-matched normal volunteers. This investigation is performed in collaboration with Dr. T. Zahn.

h. Echocardiography. Echocardiography is obtained in patients and age-matched controls to assess the presence or absence of mitral valve prolapse. These data are obtained in collaboration with Dr. R. Watson who is blind to the diagnosis of each patient or normal volunteer when echocardiography and auscultation are performed.

i. Clonidine -- An Alpha-Adrenergic Agonist. Clonidine is administered intravenously to anxious patients and volunteers to assess clinical, physiological, and neuroendocrine responses to this noradrenergic drug.

j. Urinary MHPG and Urinary Free Cortisol. Amine metabolites and urinary free cortisol are systematically evaluated using daily 24-hour urine collections across clinical state changes on and off medication.

k. Dexamethasone Suppression Test. Dexamethasone is administered to patients to evaluate the pituitary adrenal axis. Basal values are performed at baseline and at 8 a.m., 4 p.m., and 11 p.m. following dexamethasone administration.

l. Cerebrospinal Fluid and Plasma Studies. Amine metabolites, electrolytes, and peptides are also measured in blood and cerebrospinal fluid.

m. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function as well as prostaglandin-stimulated increase in cyclic-AMP are assessed in patients and age-matched normal volunteers.

n. Melatonin. Plasma and urinary melatonin is measured during clonidine and placebo infusions. Clonidine infusions will be administered to panic anxious patients and age-matched volunteers at night during sleep.

o. Glucose and Lactate Metabolism. Clinical and metabolic parameters are evaluated following the oral administration of 1.5 gm/kg glucose.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and group supportive sessions. In addition, ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. Standard medications used for the treatment of pathological anxiety may be used and include tricyclic antidepressants, monoamine oxidase inhibitors, minor tranquilizers, and beta-blockers.

c. Experimental Compounds. The anticonvulsant carbamazepine and the alpha-2 adrenergic agonist clonidine are in the preliminary stages of investigation as possible treatments of panic and phobic disorders. Imipramine and propranolol have been administered also to our panic anxious patients in order to assess whether specific biological variables correlate with predicted response to these standard antianxiety agents.

C. Major Findings

This project has been active since November 1980. A number of noteworthy findings, however, have been elucidated during the initial phase of this project.

1. Medical Illnesses and Pathological Anxiety

Detailed physical, neuropsychiatric, and laboratory evaluations have been performed in thirteen patients who met Research Diagnostic Criteria for panic disorder. None of these patients had known pre-existing medical illnesses that were thought to be related to their anxiety syndromes. Yet, within this group it has now been established that four patients have evidence of intracerebral pathology (one patient has a deep venous malformation in her right frontoparietal hemisphere; one patient has complex-partial seizures and a discrete area of decreased uptake deep in her right cerebral hemisphere; one patient has a tremor and dystonic disorder of unknown etiology; and one patient has clear evidence on computerized axial tomography of an old cerebral infarct). These preliminary findings are provocative and suggest the possibility of an increased incidence of and relationship between intracerebral pathology and some conditions of pathological anxiety. In addition, four patients had echocardiographic evidence of mitral valve prolapse, an abnormality previously found to be associated with panic attacks. Furthermore, three patients have been found to have other medical problems, including glomerulonephritis of unknown etiology, multiple endocrine adenomas, and a large uterine tumor. Several of these patients previously had received incomplete medical workups, perhaps in part because their physical complaints were exclusively attributed by physicians to anxiety. In addition, phobic avoidance may have contributed to a delay on the part of patients in seeking appropriate consultation and/or treatment.

Together, these preliminary data suggest that patients with severe anxiety require careful medical evaluations for underlying medical diseases which may mimic or exacerbate symptoms of anxiety. Furthermore, some vulnerable patients may have panic attacks triggered by a wide range of different medical illnesses. Further research is required to determine the prevalence of endocrine, cardiovascular, and neurological diseases in patients with panic and other anxiety-related syndromes. When medical illnesses are present, these patients may require specialized behavioral and/or pharmacologic and/or psychotherapeutic interventions in order for them to obtain appropriate medical care. Without appropriate treatment, some patients may be overwhelmed by phobic anxiety and avoid treatment of even life-threatening illnesses.

2. Caffeine Consumption and Pathological Anxiety

In collaboration with Drs. J.-P. Boulenger and R.M. Post, the study of both caffeine consumption and the incidence of various psychiatric symptoms has been undertaken in 16 patients with phobic disorders associated with either panic or generalized anxiety disorders. These patients were compared with 16 normal controls matched for age and sex, recruited from the patients' relatives and friends in order to control for sociocultural variables. The preliminary results of this ongoing study strongly suggest that in the patient group, but not in the control group, daily caffeine consumption (DCC) is positively correlated with the scores of trait-anxiety, as measured by the Spielberger State-Trait Inventory ($r = 0.90$; $p < 0.0001$). Although there was no correlation between DCC and state-anxiety in these two groups, various subscales of the Hopkins Symptom Checklist (HSCL-90) were positively correlated with the DCC in the patients but not in the controls. The anxiety subscale of the HSCL-90, which primarily reflects generalized anxiety, was positively and significantly correlated with DCC, but not the phobic-anxiety subscale which primarily reflects agoraphobia. These correlations suggest the existence of an increased sensitivity of the anxious patients to the effects of caffeine, particularly those symptoms related to generalized anxiety. In contrast, symptoms of agoraphobia do not appear to be influenced by caffeine consumption, a finding which supports the conceptualization of agoraphobia and generalized anxiety as distinct clinical entities.

3. Glucose and Lactate Metabolism

Four panic anxious patients have received a modified five-hour glucose tolerance test. Three patients (75%) developed anxiety and reactive hypoglycemia (glucose less than 55 MG/DL) $3\frac{1}{2}$ -4 $\frac{1}{2}$ hours following the administration of glucose. These data may be of importance in understanding the relationship between glucose and lactate metabolism and state anxiety.

4. Clonidine as a Treatment for Anxiety

Alterations in noradrenergic function have been postulated in theories of anxiety, fear, and hyperarousal states. Redmond recently proposed a model for the study of anxiety based upon the noradrenergic nucleus locus coeruleus (LC). In animals, electrical or pharmacological activation of the LC produces fear-associated behaviors and increased norepinephrine (NE) turnover, whereas lesions or pharmacological inhibition produces decreased fear-associated behav-

iors, and decreased NE as well as its metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). In man, urinary, plasma, and CSF MHPG have been correlated with state anxiety.

Clonidine, an alpha-2 adrenergic agonist that inhibits LC activity, reverses the panic anxiety associated with opiate withdrawal and decreases plasma MHPG. These findings suggested to us that clonidine might have antianxiety effects in individuals with pathological degrees of anxiety. In order to explore this hypothesis, our collaborative group (Drs. T.W. Uhde, L.J. Siever, D.C. Jimerson, and R.M. Post) have investigated the behavioral and biochemical effects of the acute intravenous administration of clonidine to 14 depressed, four panic-phobic patients, and 24 normal volunteers. Using the previously validated Spielberger State-Trait Anxiety Inventory (range 20-80), state anxiety was rated at baseline and one hour after clonidine 2 µg/kg or saline infusions. Ten of 14 depressed and all four panic-phobic patients had decreased ratings of anxiety following clonidine compared with baseline. In the combined group of depressed and panic-phobic patients, ratings of anxiety significantly decreased after clonidine (49.1 ± 2.6) compared with baseline (60.7 ± 3.2) ($n = 18$) and did not change after placebo (pre: 58.2 ± 3.3 , post: 58.1 ± 3.4) ($n = 11$). In normal volunteers, anxiety following clonidine (pre: 33.5 ± 2.3 , post: 32.2 ± 2.1 ; $n = 24$) or saline (pre: 33.8 ± 2.5 , post: 35.0 ± 2.4 ; $n = 21$) did not differ. This differential antianxiety effect of clonidine in depressed patients and not in normal volunteers was highly significant (2-way ANOVA, $p < .0001$). As predicted by a noradrenergic model of anxiety, clonidine's antianxiety effect was most potent in individuals with the highest baseline values of MHPG ($r = .44$, $p < .05$, $n = 16$, one-tailed).

In collaboration with Drs. R.M. Post and J.-P. Boulenger, preliminary data from a double-blind, clonidine-placebo crossover trial in five patients with panic disorder also suggest that ratings of generalized anxiety and panic attacks decreased after the first week of oral administration of clonidine. These decrements in anxiety were comparable to the antianxiety effects obtained following the acute, intravenous infusion of clonidine. However, four of our five patients with panic disorder developed tolerance within three weeks of chronic clonidine administration. The loss of anxiolytic effects with chronic clonidine treatment parallels the time course and development of tolerance to the inhibiting effects of clonidine on the firing rate of the LC in animals observed by others. In addition to tolerance, clonidine treatment may be associated with a number of untoward side effects such as drowsiness, sedation, and dry mouth. The role of the LC in fear-related behaviors, arousal, and the sleep-waking cycle help may explain the common association between anxiety reduction and sedation for most of the anxiolytic drugs, e.g., benzodiazepines, barbiturates, and meprobamate.

The results of these preliminary studies suggest that clonidine may have noteworthy antianxiety effects in depressed and phobic and panic anxious patients, as well as patients undergoing opiate withdrawal. In addition, our preliminary data suggest that independent measures of noradrenergic activity may be related to clonidine's antianxiety properties. Although clonidine may be less well tolerated by anxious patients than non-anxious hypertensives or patients undergoing opiate withdrawal, double-blind comparisons of clonidine to standard drug treatments, e.g., imipramine, are indicated.

5. Clonidine as an Analgesic Agent

The potential use of clonidine as a nonopioid, nonaddicting analgesic agent is of interest since clonidine has antinociceptive effects greater than or equal to morphine in animals and both blocks and reverses opiate withdrawal in man. The acute effects of clonidine 2 µg/kg on psychophysical pain has been assessed in 14 normal volunteers. Using threshold and signal detection analysis as described by Dr. M.S. Buchsbaum, preliminary data indicate that clonidine lacks analgesic activity in man as measured by the index of pain insensitivity. However, there was a significant association between fall in blood pressure and increased insensitivity ($r = 0.71$, $n = 12$, $p < .01$). The fact that the subjects with the greatest fall in blood pressure had increased pain insensitivity may suggest that, within individuals, dose-response effects of clonidine may alter nociceptive thresholds in man. Furthermore, in a subgroup of pain-insensitive individuals, clonidine produced changes on evoked response consistent with an analgesic effect. This research, accomplished in collaboration with Dr. M. S. Buchsbaum, suggests that individual differences in baseline pain sensitivity might predict patients' antinociceptive response to clonidine.

6. Clonidine and Plasma Melatonin

The effect of clonidine on plasma melatonin during sleep has been studied in collaboration with Drs. A. Lewy and L.J. Siever. Clonidine 2 µg/kg i.v. produced at least a 50% reduction in plasma melatonin in all normal controls. This preliminary finding is noteworthy and provides a unique methodology by which noradrenergic responsivity in anxious patients may be assessed.

7. Anxiety and Psychophysical Pain

The subjective experiences of anxiety and pain are prevalent symptoms in psychiatric patients. In addition, anxiety is usually thought by clinicians to enhance pain appreciation. Several authors even have suggested that anxiety intensifies psychophysical pain by directing attention toward pain sensations. Furthermore, alterations in noradrenergic function have been implicated in the modulation of both anxiety and pain. In an attempt to clarify these variables, we have investigated the relationship among anxiety, psychophysical pain, and plasma-free MHPG in 12 normal volunteers.

An index of pain insensitivity was obtained 1½ hours after the blind administration of a placebo infusion that had been randomly paired with an active medication. Each subject received 93 randomly presented shocks (1-31 mA) to the forearm. Subjects rated each sensation as noticeable, distinct, unpleasant, or very unpleasant. An index of pain insensitivity, derived from the subject's ability to discriminate between distinct and unpleasant sensations, was obtained by threshold and signal detection analysis. Increases in this measure are associated with morphine and aspirin analgesia in normal volunteers.

In collaboration with Drs. M.S. Buchsbaum and D.C. Jimerson, we have demonstrated significant correlations between state anxiety and scores on the insensitivity index ($r = .67$, $n = 12$, $p < .05$) and state anxiety and plasma-free MHPG ($r = .59$, $n = 12$, $p < .05$). Thus, the subjects with the highest ratings of state anxiety had the greatest plasma-free MHPG and were least able to discrimi-

nate between distinct and unpleasant sensations. By median split, the six most pain insensitive individuals had significantly higher levels of plasma-free MHPG ($3.2 \text{ ng/ml} \pm 0.2 \text{ S.E.}$) and anxiety (39.1 ± 1.8) compared to the six least pain insensitive individuals (MHPG 2.5 ± 0.1 , $p < .20$; anxiety 27.8 ± 1.6 , $p < .001$).

These findings (presented at the Annual Meetings of the American College of Neuropsychopharmacology and the American Psychiatric Association) represent one of the first reports to suggest that high anxiety may reduce, rather than enhance, the ability to discriminate the amount of pain a patient experiences. In some circumstances, therefore, treating pain patients with anxiolytics might paradoxically intensify the amount of pain experienced as measured by the insensitivity index.

8. Anxiety and Sleep Architecture

Insomnia is commonly believed to result from anxiety or other states of increased autonomic arousal. In accordance with this hypothesis many clinicians have employed relaxation techniques, biofeedback, systematic desensitization, and antianxiety pharmacotherapy in the treatment of insomnia. Although anxiety is a frequent concomitant of insomnia, no laboratory has investigated either the prevalence of insomnia or the sleep architecture of patients who meet Research Diagnostic Criteria (RDC) for the panic and phobic disorders. Furthermore, a comparison of sleep between panic anxious and depressed patients, as well as normal controls, is indicated since both panic anxious and depressed patients respond to tricyclic and MAO inhibitor drugs. In collaboration with Drs. J.C. Gillin and R.M. Post, we are investigating both the frequency of complaints of insomnia and the sleep architecture of patients who meet RDC for panic or phobic disorders. Preliminary evidence suggest that many of the panic anxious patients do not report subjective sleep disturbance. In fact, several of the patients stated that the subjective experience of restful sleep was associated with less daytime anxiety and reduced number of panic attacks after awakening. While these particular patients "looked forward" to sleep onset, they also recorded numerous sleep-incompatible and avoidance behaviors, and often delayed retiring to their rooms until after midnight. In relationship to sleep architecture, there was no significant difference between anxious patients and the published norms of age-matched normal volunteers in any of the following criteria: early morning awakening, sleep efficiency, REM latency, intermittent awake time, delta sleep, and REM density. Preliminary findings suggest, however, that the panic anxious patients as a group had significantly less total sleep time, even though they did not experience subjective sleep disturbance.

D. Proposed Course of Project

Research conducted by the Section on Psychobiology has demonstrated a blunting of the clonidine-induced growth hormone (GH) response in affectively ill patients compared to age- and sex-matched controls. These findings, which have been replicated by four independent research groups, may suggest decreased postsynaptic noradrenergic function in endogenous depression.

Increasing evidence also suggests a heterogeneity in anxiety and depressive syndromes. Thus, the neuroendocrine (GH) response to clonidine in patients with anxiety disorders (with and without depression) may provide a useful methodology

for elucidating the biological relationships of various anxiety disorders to each other as well as to depression. In addition, we plan to challenge patients with yohimbine, an alpha-2 adrenergic antagonist, in order to further assess noradrenergic function in patients with pathological anxiety and depression. Diazepam, a standard antianxiety agent, will also be given to patients and controls to investigate the relationships among anxiety, psychophysical pain, and various peripheral correlates. We also intend to explore the mechanisms involved in caffeine-induced anxiety by administering caffeine to anxious patients and normal controls in a placebo-controlled study. Further delineation of the clinical response to caffeine is indicated because caffeine consumption is correlated with symptoms of generalized anxiety in patients with panic attacks, but not in normal volunteers. Caffeine derivatives also activate noradrenergic activity in animals when iontophoretically applied to the LC. Furthermore, caffeine has been shown by others to antagonize the biochemical and pharmacological effects of benzodiazepines in humans. This study, therefore, will represent an initial attempt to investigate the effect of caffeine on mood, anxiety, and behavior in clinically distinct patient groups.

Studies of the clinical efficacy of clonidine, carbamazepine, imipramine, and propranolol in panic and phobic patients have just been initiated. Our preliminary research with clonidine in depressed and anxious patients and normal volunteers is encouraging and suggests that clonidine might be especially useful in patients who experience context-dependent, e.g. anticipatory anxiety. In addition, carbamazepine will be studied with special emphasis on its potential usefulness as a prophylactic agent in blocking spontaneous panic attacks. These clinical trials, in conjunction with concomitant measurements of the neurotransmitter effects, should enhance our understanding of alterations in neurotransmitter pathways associated with pathological anxiety and its amelioration with appropriate psychopharmacotherapies.

E. Significance to Biomedical Research and the Program of the Institute

Several epidemiological surveys have suggested that pathological degrees of anxiety may adversely influence a large segment of our population. Agoraphobia, an anxiety syndrome associated with "spontaneous" panic attacks, results each year in the impairment of individuals previously well-functioning and productive. Furthermore, the role of anxiety and stress in coronary heart disease has been suggested by a number of studies. An improved understanding of the clinical and biological aspects of both normal and pathological anxiety is thus critically needed. Nonetheless, anxiety disorders and related syndromes may be among the least understood of the endogenous psychiatric illnesses. It is hoped that the developing battery of clinical and biological tests in patients with anxiety and related disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00072-02 BP | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 1, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Psychophysiological Investigation of Multiple Personality Disorder | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.: F.W. Putnam, M.D.</td> <td style="width: 33%;">Staff Psychiatrist, Sect. on Psychobiology</td> <td style="width: 33%; text-align: right;">BP NIMH</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>OTHER: R.M. Post, M.D.</td> <td>Acting Chief</td> <td style="text-align: right;">BP NIMH</td> </tr> <tr> <td>M.S. Buchsbaum, M.D.</td> <td>Chief, Sect. on Clinical Psychophysiology</td> <td style="text-align: right;">BP NIMH</td> </tr> <tr> <td>L.J. Siever, M.D.</td> <td>Senior Staff Fellow</td> <td style="text-align: right;">CN NIMH</td> </tr> <tr> <td>E.K. Silberman, M.D.</td> <td>Staff Psychiatrist</td> <td style="text-align: right;">LPP NIMH</td> </tr> <tr> <td>T.P. Zahn, Ph.D.</td> <td>Research Psychologist</td> <td style="text-align: right;">LPP NIMH</td> </tr> <tr> <td>H. Weingartner, Ph.D.</td> <td>Chief, Unit on Cognitive Studies</td> <td style="text-align: right;">LPP NIMH</td> </tr> </table> | | | P.I.: F.W. Putnam, M.D. | Staff Psychiatrist, Sect. on Psychobiology | BP NIMH | | | | OTHER: R.M. Post, M.D. | Acting Chief | BP NIMH | M.S. Buchsbaum, M.D. | Chief, Sect. on Clinical Psychophysiology | BP NIMH | L.J. Siever, M.D. | Senior Staff Fellow | CN NIMH | E.K. Silberman, M.D. | Staff Psychiatrist | LPP NIMH | T.P. Zahn, Ph.D. | Research Psychologist | LPP NIMH | H. Weingartner, Ph.D. | Chief, Unit on Cognitive Studies | LPP NIMH |
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| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Psychobiology | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) <p> This project investigates the syndrome of <u>multiple personality disorder</u> (MPD). The investigation focuses on three major areas. The first is the physiologic and <u>neurophysiologic differences</u> reported to exist among the alternate personality states. The existence of certain types of differences has been documented and further research is underway to elucidate the mechanisms of these state-related changes. The second focus is on the symptoms, presentation, and <u>phenomenology</u> of the clinical syndrome. An extensive case finding study is underway, sampling cases in treatment in the United States and abroad. The third focus is on a prospective study of cases currently in treatment to clarify the long-term <u>outcome</u> of this unusual disorder. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | |

I. Project Description

A. Objectives

This study is a multidisciplinary effort to investigate and rigorously document the clinical and physiological phenomena of the multiple personality disorder (MPD) syndrome. There are three major strategies currently being employed to pursue this goal.

1. The first investigative line of approach utilizes repeatable neurophysiological measures to investigate differences among personality states, compared to those in normal controls who are asked to simulate alternate personality states. Repeated measures are determined on each personality state in both patients and controls, and the degree of repeatability and variability are assessed.

2. The second line of inquiry is the development of a large case study data base from which to assess the nature of the syndrome as it presents in clinical settings. The emphasis of this study is on the presentation, diagnosis, clinical symptomatology, treatment, and outcome of this disorder. The data for this study are derived from two sources, the clinical literature and a large scale questionnaire study directed at cases currently in treatment in the continental United States. A smaller scale questionnaire study sampling foreign cases for purposes of comparison is also underway.

3. The third avenue of investigation is the development of a cohort of patients to follow through treatment to assess the effectiveness of various therapeutic modalities and the long-term outcome and prognosis of the disorder. Included in this cohort of identified cases are young children, identified in cooperation with two area protective services.

B. Methods Employed

1. Subjects

a. Patients who meet DSM III criteria for MPD are admitted as inpatients or outpatients to the 3-West Clinical Research Unit, Section on Psychobiology, Biological Psychiatry Branch. These patients serve as subjects in the neurophysiological studies.

b. Patients in treatment in the Maryland, Virginia, and D.C. area are screened and interviewed for participation in the long-term follow-up study.

c. Normal volunteers and professional actors serve as control subjects.

2. Neurophysiological Evaluation

a. Drug-free subjects undergo a repeated series of two lead visual averaged evoked response trials. The experimental design uses the augmen-

tation/reduction paradigm developed by Dr. M. S. Buchsbaum. Each alternate personality state is tested on five separate days to measure the stability of the evoked response. At least three alternate personality states are tested per subject. Each patient is compared to an age- and sex-matched control.

b. EEG spectral power mapping studies, using the RCBEAM technology are carried out on all alternate personality states studied in with the AER. At least six trials are run on each personality of each patient and matched control.

The above studies are in collaboration with Dr. M.S. Buchsbaum.

c. Galvanic skin response (GSR) and autonomic measures such as respiration, temperature, and heart rate are studied across the alternate personality states of patients and simulating controls in collaboration with Dr. T. Zahn. Each personality is studied on five separate days. An autonomic conditioning paradigm is used to assess the independence of learning and habituation across personality states.

d. Proactive inhibition memory testing developed by Drs. E.K. Silberman and H. Weingartner is used to measure the transfer of learning and memory across personality states and assess the degree of the clinically reported amnesia, which is a core symptom of this disorder.

3. Questionnaire Study

a. An extensive (24 page) computer-coded questionnaire derived from clinical experience and a systematic review of the relevant literature was developed. The questionnaire was reviewed by the Epidemiology Branch of NIMH and underwent one year of pilot trials. The questionnaire is now being distributed to two groups of clinicians. The first group is a compiled mailing list of therapists known to be treating patients with MPD. The second group consists of clinicians chosen from the American Psychiatric Association mailing list. A total of at least 1000 clinicians will be surveyed in the course of this study. A third group being surveyed consists of approximately 50 foreign physicians known to be treating patients with MPD.

4. Prospective Studies

a. The first prospective study involves the following of a cohort of adult MPD patients from the Maryland, Virginia, and D.C. area. These patients were all seen in consultation with their primary therapist and underwent a standardized, videotaped interview. These patients will be followed at yearly intervals throughout the course of their treatment.

b. The second group of patients involved in the prospective studies are children identified with the help of two local sexual abuse agencies, the Prince Georges County Sexual Assault Center, and the Montgomery County Protective Services. These children fit a profile developed and distributed to caseworkers of these two agencies. The focus on sexually abused children derives from the data obtained in the questionnaire and literature review studies, which show an extremely high correlation between sexual abuse from ages 1-10 years and the development of MPD by adulthood.

C. Major Findings

1. Neurophysiology

a. Two lead visual evoked response studies show statistically significant differences in 26 measured variables in the multiple personality patients compared to their matched controls. These significant differences include both differences in amplitude and in latency. A total of 11 patients and 10 matched controls have been studied to date.

b. Power spectral mapping studies have been completed on 10 patients and 10 matched controls. These studies show statistically significant differences among certain types of alternate personality states, e.g., obsessive-compulsive alternate personalities, compared to primary personalities or controls. These findings parallel very closely the observed differences between obsessive-compulsive patients and normal controls reported by other investigators.

c. GSR and other autonomic measures are found to be statistically significant and different across alternate personality states. Interesting changes in heart rate and skin temperature, which are personality state specific have been repeatedly observed in individuals with MPD, compared to simulating normal controls.

d. The proactive inhibition memory testing paradigm demonstrates that the exchange of task-specific memory across personality states is variable, and often shows a polarized, directional property. The amount of memory transfer across personalities is significantly decreased compared to simulating normal volunteer controls.

2. Questionnaire Study

The questionnaire study is currently underway. The initial return rate on over 200 questionnaires is over 30%. A sampling of at least 100 current cases is considered feasible. The preliminary results bear out many of the hypotheses derived from the literature review study, including the extremely high correlation between a history of childhood sexual abuse and the subsequent development of MPD. Several other findings are emerging from the data, including an interesting association of MPD with an anorexia nervosa-like syndrome, and the incidence of MPD in children of concentration camp victims.

3. Prospective Studies

No findings are currently available from these studies which were initiated in the last 18 months. There are 32 patients and therapists participating in this ongoing project currently.

D. Significance to Biomedical Research and to the Program of the Institute

The physiological studies are confirming the often reported anecdotal reports of physiological differences among alternate personality states. These physiological differences are replicable on repeated testing and are not present in the simulating control subjects. These studies may provide a model with which to understand the physiology of psychosomatics, i.e. how personality interacts with bodily processes.

The questionnaire study is delineating the specifics of the syndrome, which will be of use to mental health professionals. There is growing evidence that many of these patients are misdiagnosed and, consequently, mistreated. Clarification of the core symptom set of this disorder may reduce this problem.

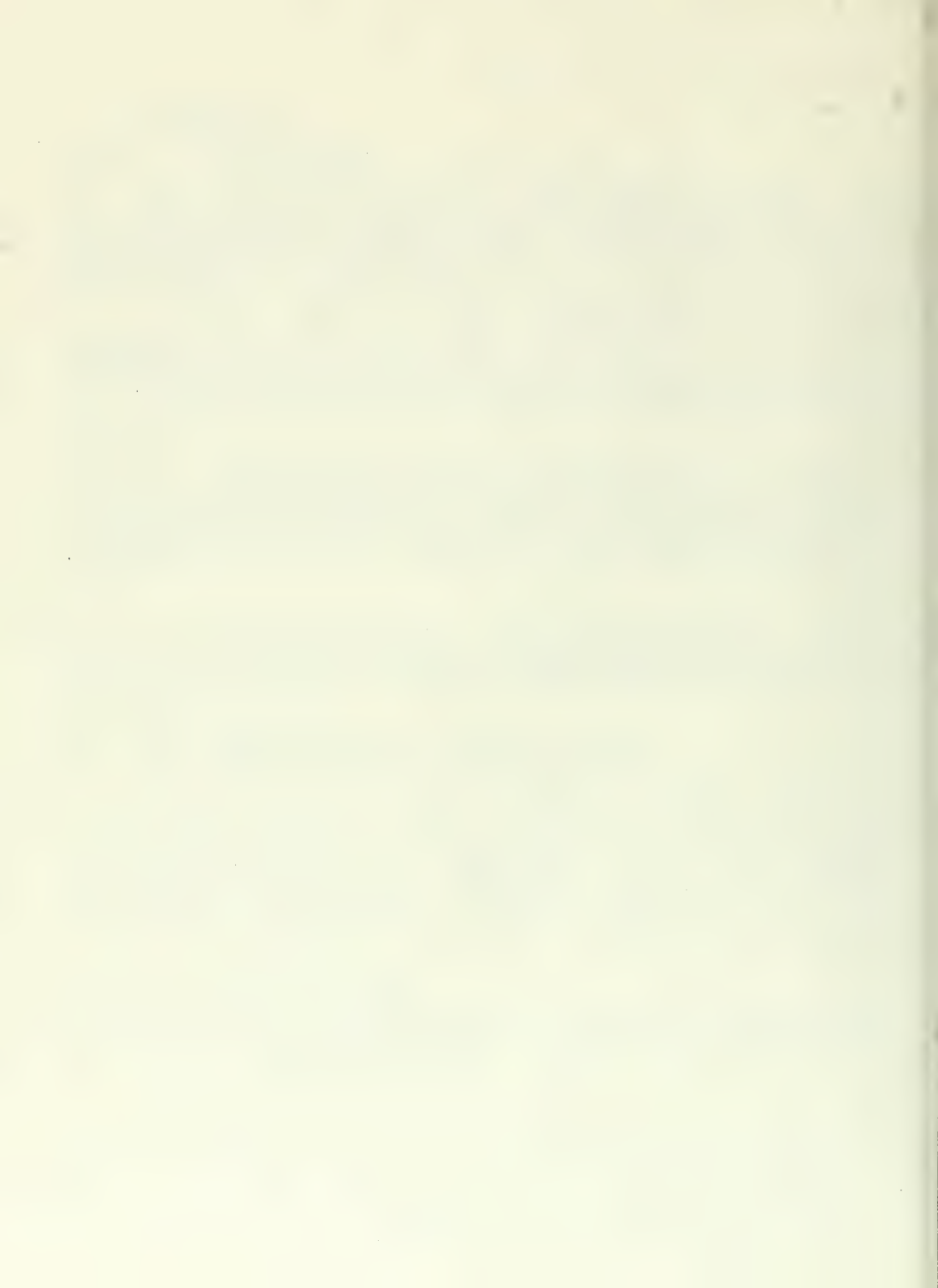
Multiple personality disorder may be a far more prevalent syndrome than has generally been recognized. The linkage of this syndrome to child abuse is becoming more apparent and suggests a preventative intervention. The physiological differences that can exist across the different alternate personality states have interesting implications for the understanding of mind/body interactions and the relationship of physiological alterations to the development of psychopathology.

E. Proposed Course of Project

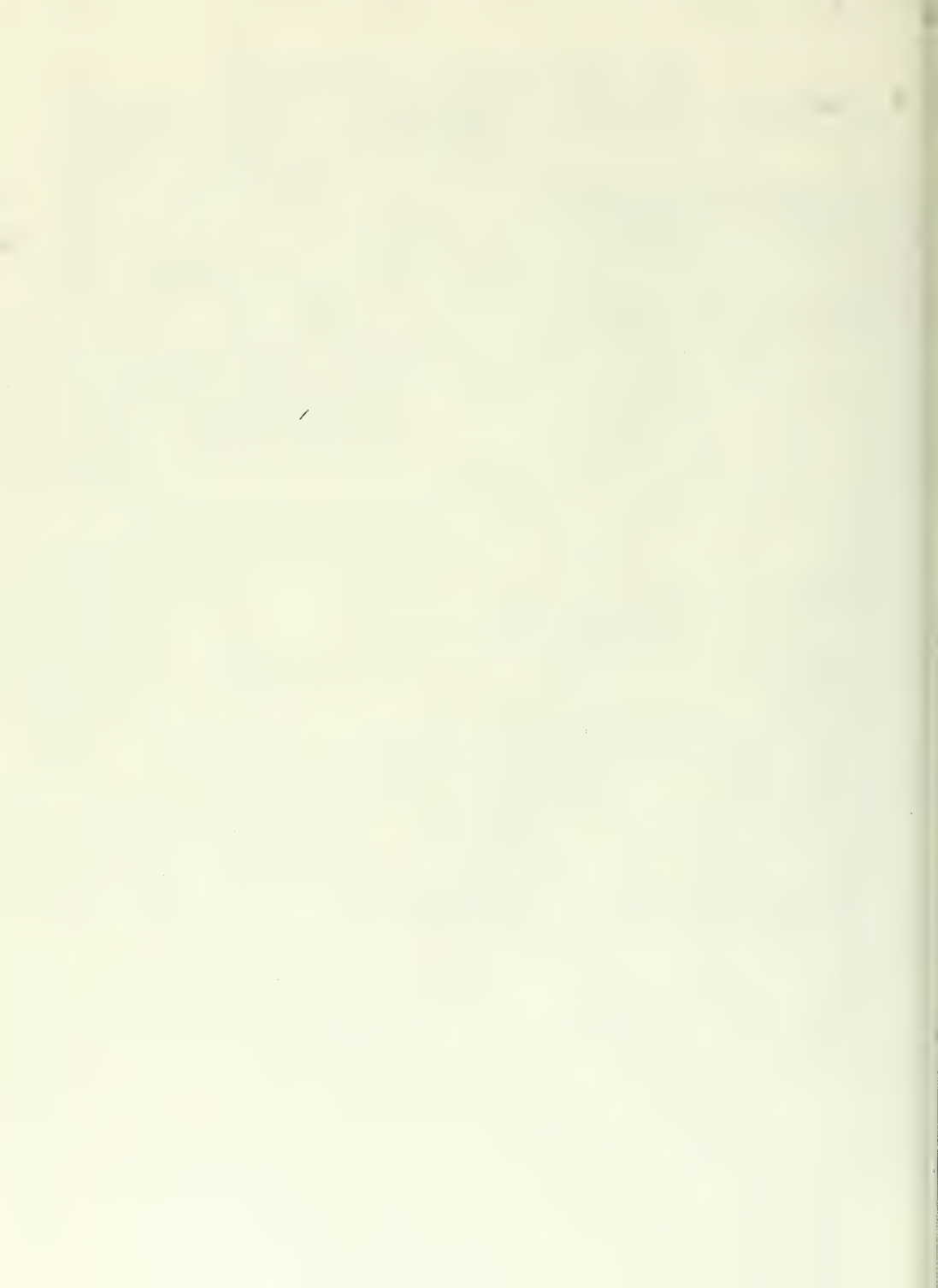
1. The neurophysiological studies will be continued in an effort to determine the biological mechanisms underlying the shifts in evoked potentials associated with changes in personality state.

2. The large scale questionnaire study will serve to develop a symptom profile of this disorder. Relative efficacy of treatment methods will be determined by the questionnaire follow-up.

No publications to date.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00049-07 BP |
| PERIOD COVERED October 1, 1982 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Psychosocial Factors in the Prognosis of Melanoma | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> PI: Daniel P. van Kammen, M.D., Ph.D. OTHERS: G.N. Rogentine, M.D. Bernard H. Fox, M.D. </div> <div style="width: 50%;"> Unit Chief, Sec. on BP NIMH Neuropsychopharmacology Staff, Georgetown University Washington, D.C. Staff, Field Studies and Statistics Program NCI </div> </div> | | |
| COOPERATING UNITS (if any) Field Studies and Statistics Program, NCI | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Neuropsychopharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 0.8 | PROFESSIONAL: 0.6 | OTHER: 0.2 |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> <div style="width: 30%;"> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div style="width: 30%;"> <input type="checkbox"/> (c) NEITHER </div> </div> | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <div style="text-align: center; padding-top: 20px;"> <p>This project has terminated.</p> </div> | | |



| | | |
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Project Description

The Section on Neuropsychopharmacology (1) plans and conducts multidisciplinary research on the etiology and treatment of schizophrenia and (2) conducts research to define the biological dysfunction in schizophrenia, particularly through repeated drug-free evaluations.

Objectives

1. Multidisciplinary research on the etiology, treatment and prevention of schizophrenia.
2. Evaluation of the dopamine and other amine and peptide hypotheses of schizophrenia.
3. Evaluation of potential markers of vulnerability and predictors of antipsychotic drug response.
4. Psychopharmacological and biochemical evaluation of trait- and state-dependent variables of schizophrenia.
5. Evaluation of novel treatments such as plasmapheresis, hemodialysis, propranolol and naltrexone.

Methodology

One to 14 days after admission in a double-blind fashion, patients are taken off their neuroleptic medication and put on placebo. After this two-week "washout period" patients go through a 3- to 4-week test period in which diagnostic evaluation, medical and neurological workup, cerebrospinal fluid (CSF) collections, drug infusions (amphetamine, beta-endorphin, placebo), psychological and psychophysiological testing and neuroendocrine evaluations take place. These are followed by two or more drug trials which include pimozide and propranolol. During any of these drug trials, testing similar to the admission test week can take place. Each drug period is separated by a two-week placebo "buffer" period. The last of these periods is either followed by another test period or immediately followed by a 4-week clinical period where, if indicated, the patient is put on generally available antipsychotic medication and prepared for discharge. This extensive data collection system allows us to evaluate the individual variables and drug responses against one another to observe the patient in different clinical states. It enables us to develop psychosocial, endocrine, biochemical and pharmacological profiles on patient groups defined by each variable.

A major problem of schizophrenia research has always been certainty of diagnosis. In addition to using the International Pilot Study of Schizophrenia criteria developed by the World Health Organization and the former Psychiatric Assessment Section of the Adult Psychiatry Branch, we are applying the Research Diagnostic Criteria (RDC) described by Dr. Spitzer and his collaborators to diagnostically define our patient population. This year we have added the DSM III criteria.

The training of the 4-East nursing staff in the application of the Bunney-Hamburg psychosis, depression and mania scale continues. We have added a scale to measure sedation. The Brief Psychiatric Rating Scale (BPRS) modified by the NIMH behavior rating scale committee under Dr. D.L. Murphy was adopted for biweekly ratings by our nursing staff and is used weekly by "blind" psychiatrist-raters.

Major Findings: PharmacologicalA. Dopamine Stimulation (d-Amphetamine)

d-Amphetamine, an indirect dopamine receptor agonist, has been shown to worsen some acute psychotic symptoms. At the same time, numerous clinical reports of increased social contact and therapeutic effects have appeared in the psychiatric literature over the last 25 years. This, therefore, is a potentially crucial area of investigation for clarifying the limits and nuances of the dopamine hypothesis. Patients receive i.v. amphetamine and i.v. placebo during the initial test period. This is repeated during pimozide, pimozide withdrawal and lithium treatment.

Schizophrenic patients appear to respond differently to amphetamine than do depressed patients, although there is heterogeneity within each group. Strikingly, remitted patients do not become more psychotic; only 18 of 45 schizophrenic patients showed increased psychotic symptoms. However, within this schizophrenic group the more severely psychotic patients improved (N = 13) while some patients in early remission got worse. Our data support the concept that the amphetamine-induced worsening is an "episode marker". Preinfusion spinal fluid MHPG correlated significantly with the change in psychosis. Pimozide decreased the amphetamine-induced worsening, but the activation-euphoric cluster was unaffected. Lithium pretreatment attenuated the activation and euphoric effects but not the psychotogenic effect. Worsening following d-amphetamine administration predicted early relapse after pimozide withdrawal. Amphetamine response predicted the 4th and 5th week of pimozide response and the 3rd week of antidepressant response in post-psychotic, depressed, schizophrenic patients and the small antipsychotic effect of lithium. No increased behavioral response was observed following amphetamine administration during the pimozide withdrawal phase (dopamine receptor supersensitivity).

We replicated our previous finding that prolactin levels were increased 25 and 40 min. post-amphetamine infusion. This was rather striking since the placebo infusions which took place at the same time of the day, i.e., between 8 and 9 AM, showed a slight decrease from the rise of prolactin during sleep as a result of diurnal variation. This is in contrast to the expected decrease in prolactin levels following increased dopamine activity. The increase is hypothesized to be due to stress or activation of serotonin mechanisms in the hypothalamus and pituitary. Amphetamine raised cortisol levels with and without naltrexone pretreatment. However, contrary to what was reported in normal volunteers, beta-endorphin values did not correlate with control values. Also, following the placebo infusion, beta-endorphin (IR) plasma levels rose. Several papers are in press. The data has been presented at the Annual Meeting, American College of Neuropsychopharmacology.

B. Dopamine (DA) Infusion

In collaboration with Drs. Zis, Gold, Yen and DeFraités, dopamine (DA) infusions were used to test the prolactin, growth hormone (GH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in schizophrenic patients during drug-free, neuroleptic-medicated and neuroleptic withdrawal phases. Dopamine does not cross the blood-brain-barrier and acts, presumably, at the DA terminals of the pituitary and median eminence. Preliminary data indicate that schizophrenic patients may have an abnormal LH response and a supersensitive prolactin response to DA in the neuroleptic withdrawal phase. Three papers are in preparation.

C. Apomorphine

Apomorphine (0.75 mg) leads to an increase in growth hormone. Poor premorbid functioning was associated with a blunted GH response. The GH response correlated negatively with platelet MAO activity. Two papers have been submitted by Dr. Malas.

D. Dopamine Receptor Blockade and Schizophrenia

Pimozide in acutely psychotic patients: Following a washout period, patients are started on a double-blind, placebo-controlled trial of the effects of pimozide in acutely psychotic and remitted patients. This antipsychotic agent is of special interest because of its relatively specific postsynaptic DA receptor blocking effect.

During this project endocrine studies and CSF studies are conducted. Selected patients are put on a sleep study to evaluate the effects on sleep. Data are being analyzed.

Other studies include endocrine studies, cyclic AMP in CSF, lack of effect of pimozide on DBH in spinal fluid, metabolites of DA, norepinephrine (NE) (decrease) and serotonin in the spinal fluid and 24-hr. urine collections in addition to the standard battery of behavioral ratings. In collaboration with Dr. Heykants of Beerse, Belgium, we are measuring pimozide levels in our patients with a radioimmunoassay. These levels will be examined in relation to the above-mentioned hormones and urinary free cortisol. We are now exploring CSF and endocrine variables at different time-points of the pimozide trial, including during the withdrawal phase with apomorphine examined.

E. Lithium in Schizophrenia

A review of the literature and the Section's contribution is being written.

Major Findings: Biological

A. Gamma-Aminobutyric Acid (GABA) and the Dopamine Hypothesis of Schizophrenia

1) GABA levels in CSF of schizophrenic patients have been studied in collaboration with Dr. Hare. In 30 drug-free schizophrenic patients, GABA levels were not significantly lower than in controls. However, female patients (30 years) had significantly lower levels than age-matched female controls. Levels increased with duration of illness, number of hospitalizations and months of hospitalization. This suggests that with time the low levels are being compensated.

2) Diazepam, one of the first compounds found to increase GABA function in the brain, was studied in our patients in high doses up to 300 mg/day. We administered diazepam in doses higher than 40 mg/day to five patients. Only two severely psychotic patients received 300 mg of diazepam with equivocal results. One patient, who was still relatively nonpsychotic following pimozide withdrawal, became acutely psychotic when he was withdrawn from 70 mg of diazepam. In the more psychotic patients we did not observe side effects such as ataxia and sedation. A paper has been published (Dr. Jimerson).

3) A paper about the effect of gamma-hydroxybutyrate (GHB) in schizophrenia has been published (Dr. Schulz). A GHB-associated improvement was observed in two

patients. In general, GHB activated schizophrenic symptoms. Low platelet MAO, high CSF HVA and an augmentation with AER technique suggested improvement was present. This project has ended.

B. Monoamine Oxidase (MAO)

MAO in plasma or platelets has been implicated in vulnerability to chronic unremitted schizophrenia and affective disorders. We are presently evaluating the relationship between clinical and biochemical variables and activity levels. Our male patients appeared to have significantly lower MAO activity than normal controls. We reported similar preliminary findings in a small group of patients. Low MAO activity was associated significantly with elevated CSF NE, good premorbid functioning, elevated GH response to apomorphine and high CSF tyrosine hydroxylase co-factor activity. The association between low MAO activity and good premorbid functioning was also observed in 85 patients.

C. Endocrinological Project

Endocrine studies remain a major investment of the Section.

1) Evaluation of baseline hormonal levels in schizophrenic patients is being conducted, focusing on hormones that are under total or partial control of DA. Prolactin, GH, FSH and LH were measured in serum. Measurements thus far have been made with placebo, chronic pimozide, GHB, propranolol and naltrexone. Increases in prolactin levels followed DA blockade (by pimozide) as expected, but no effects were observed with (chronic) propranolol (for the males).

2) We also studied the GH and prolactin response to apomorphine in drug-free psychotic patients. Patients participated three days after neuroleptic withdrawal, three weeks after placebo. Poor premorbid patients had significantly lower responses than controls and "reactive" schizophrenic patients.

3) Urinary free cortisol (UFC) levels in drug-free patients and in patients on opiate and pimozide and on the antagonist naltrexone are being examined. In contrast to the report by Drs. Chen and Carroll, some schizophrenic patients do seem to have increased levels (like depressed patients). This has led us to study the dexamethasone suppression test early in the psychotic episode. Chronic naltrexone had no effect on UFC in a small group of patients. However, plasma cortisol levels were increased in a larger group. Pimozide seemed to normalize UFC levels. During pimozide withdrawal a further significant decrease in UFC was observed (paper in preparation).

4) Chronic pimozide treatment increases serum prolactin and decreases LH. A paper has been published.

5) Hypertonic saline infusions have been started in drug-free and medicated patients (Drs. Malas and Gold) to study vasopressin response.

6) Amphetamine and placebo infusions increased plasma radioimmune beta-endorphins (with Drs. Schulz and Pickar). The effect was not significantly different between the two infusions, but both infusions led to a significant increase in beta-endorphin (IR) levels.

D. Cerebrospinal Fluid Studies (CSF) in Schizophrenia

CSF studies for c-AMP and c-GMP mean values were not different from depressed comparison groups of patients. Probenecid increased the baseline values. The effects of pimozide are presently under study.

A study of pterine cofactor activity in our schizophrenic patients and in normal comparison groups has been published (J.D. Garbutt, R. Levine, W. Lovenberg and J. Ballenger). Schizophrenic patients had levels similar to those of 20 normal controls.

Evaluation of peptides such as vasopressin, vasotocin, oxytocin, angiotensin I and II, somatostatin and endorphin-like activity in CSF has begun. Vasopressin levels were lower in the schizophrenic patients ($p < .05$). For evaluation of CSF GABA see above; for CSF NE, DBH and endorphins, see below.

With Dr. Linnola, we are measuring NE, DA, epinephrine, MHPG, HVA and 5HIAA in the CSF of our patients. NE and DA were significantly correlated. 5HIAA was lower in seven patients with severe suicidal tendencies or who later suicided than in those seven who were matched for age, sex and height and who had never made suicide attempts. This is consistent with other reports in the literature of depressed patients. Low 5HIAA may be associated with impulsiveness, aggressiveness and violence rather than with depression per se (Dr. Ninan).

DBH in CSF of schizophrenic patients showed levels similar to that of normals, but a subgroup of good premorbid (reactive) patients appeared to have lower levels. Patients on pimozide became less psychotic relative to the decline in CSF NE ($r = .71$, $p < .02$). Several papers have been published.

Our male drug-free patients appeared to have low or normal radioimmune beta-endorphin and radioreceptor opioid activity in the CSF. Several papers have been published (Drs. Pickar and Naber).

E. Norepinephrine (NE) Systems

1) Dr. Marian Kafka measured alpha-receptor activity in our drug-free patients. Although the alpha₂-receptor binding is not different in schizophrenic patients from normal controls, 10% of the patients had very high levels. In the male patients c-AMP production was 50% lower than in the controls in response to prostaglandin stimulation. This decrease seems to be related to decreased activity in adenylyl cyclase. The male patients had poorer premorbid functioning than the female patients, which may explain the sex discrepancy. In the small number of subjects in whom c-AMP response was measured during neuroleptics, lithium or propranolol produced no change in values. In depth study of adenylyl cyclase in a few subjects is underway. Several papers have been published.

2) Dr. Tallman measured beta-adrenergic receptors on leukocytes from psychotic and nonpsychotic patients during drug-free treatment and during high dose dl- and dl-propranolol administration. There appears to be no difference between schizophrenic patients and normal controls while the receptor count stays stable over time. We are now analyzing the data in our propranolol treatment study.

3) The clinical evaluation of d- and dl-propranolol with concomitant measurement of plasma levels, beta-receptor binding, endocrines and CSF metabolites has been discontinued. A few patients improved. Data analysis is underway.

F. Endorphins

1) A study with a longer-acting opiate antagonist, naltrexone, has been completed. Data analysis is underway.

Major Findings: Physiological - Anatomical

A. Laterality Hypothesis

All patients are tested with the Luria test for lateralized sensory and motor function. Data will be compared to drug response and CAT scan abnormalities (Lee Mann).

B. CAT Scan Abnormalities

CAT scan abnormalities have been found in about 30% of our patient population, independent of drug use and duration of illness. Psychological and biological exploration of this finding is underway.

C. Positron Emission (PET) Scans

This new method of evaluating brain function has been started in eight drug-free patients with Drs. Buchsbaum, Ingvar and Kessler. A first paper of eight patients has been published. A second series is underway. Schizophrenic patients appear to have decreased cortical frontal metabolism.

D. EEG Recording of Sleep

Hemodialysis does not seem to affect sleep in our patients. With Drs. Mendelson, Gillin and Buchsbaum, we have begun 16-lead EEG sleep recordings to "map" the EEG frequency in relation to underlying brain structure in drug-free schizophrenic patients.

E. Psychophysiological Testing

1) Dr. Zahn of the Laboratory of Psychology and Psychopathology is testing our patients during drug-free treatment as well as during different drug conditions. Data in relationship to amphetamine response, to neuroleptic response and to biological variables are being analyzed.

2) Pain testing and average evoked response (AER) potential studies are being conducted by Dr. Buchsbaum.

Major Findings: Behavioral and Psychological

A. Plasmapheresis

Eleven patients underwent plasmapheresis (real or sham) three times per week for two weeks. Five underwent sham and six underwent real plasmapheresis. None of the patients improved. One patient worsened following discontinuation of active plasmapheresis. The study was based upon an autoimmune hypothesis of schizophrenia. Immunoglobulins are clearly lowered to 10% of pre-pheresis values with this procedure. Furthermore, this study was an extension of the negative hemodialysis results. Dr. Schulz presented this study at the Early Clinical Drug Evaluation Unit

meeting in Florida, May, 1982. Dr. Papadopoulos measured the plasma and CSF immunoglobulins and Dr. Klein of the Clinical Center Bloodbank supervised the plasmapheresis. Our data do not support a protein-bound circulating schizophrenogenic substance.

B. Information Processing Project

1) A simply administered, objective, quantified measure of cognitive disorder has been developed. Preliminary results have disclosed a highly significant difference between patients and normals. We will use this technique to investigate the effects of psychoactive drugs and state variables on schizophrenic thought disorder. We are also investigating the presence and degree of thought disorder in the families of schizophrenic patients.

2) Schizophrenic thought disorder is also being investigated with a battery of psychological tests mainly derived from standard I.Q. tests; some scores appear to be impaired in schizophrenia. This emphasizes the need for a special psychological evaluation of our patients.

3) Evaluation of memory and cognition in schizophrenia is underway (Dr. Weingartner).

Major Findings: Electrophysiology

Dr. Hommer initiated single-cell recordings from DA neurons in the substantia nigra. Extensive evaluation of the interactions between the opioid and DA systems resulted. A paper was presented at the Neurosciences Meeting (1982) and is being submitted for publication.

Significance to Biomedical Research and the Program of the Institute

These studies contribute to the understanding of schizophrenia. Our work integrates the multidisciplinary approaches to this disorder.

Hemodialysis: The claims of miraculous cures (1977) with this technique are under investigation throughout the world. To date, there are many places in this country where hemodialysis is already being used as a treatment for schizophrenic patients. Our double-blind evaluation study did not show improvement in our patients. Today it is clear that dialysis is ineffective and should not be used.

Plasmapheresis: Although immunoglobulins were reduced to 10% of pre-pheresis levels, no improvement was observed in the six patients who underwent active plasmapheresis. This finding decreased the likelihood of finding an immunological disorder in schizophrenia.

Lithium: Lithium can be of help in the management of schizophrenic patients, although it is less effective than neuroleptics. It may have antidepressant properties in post-psychotic depression. Amphetamine effects predicted these drug responses in schizophrenic patients. This finding decreases the likelihood that lithium response indicates an affective disorder diagnosis.

Plasma and CSF DHB was lower in some good premorbid, neuroleptic-responsive schizophrenic patients. CSF NE was higher than in normal controls but levels decreased with neuroleptic-induced improvement. NE correlated with the age of onset in female patients and inversely with platelet MAO activity.

Platelet MAO Activity: Although lower in our male patients compared to controls, low MAO activity seems to be associated with good premorbid social functioning rather than with schizophrenia.

Prostaglandin E₂-stimulated c-AMP levels were lower in schizophrenic males and females compared with normal controls. This may implicate prostaglandins in schizophrenia and also suggests impaired neuronal functioning. Alpha-receptor binding was increased in 10% of our drug-free schizophrenic patients.

Schizophrenic patients as a group appeared to be more pain tolerant than normal controls. This could be reversed by naltrexone, which may imply a disturbance in endorphin regulation of pain mechanisms in schizophrenia. CSF endorphins (measured by radioreceptor and radioimmunoassay) were not elevated in our schizophrenic patients.

Perception of Genetic Risk for Schizophrenia by Relatives of Schizophrenic Patients: Dr. S.C. Schulz and P. Schulz, MSW, interviewed relatives of patients for their understanding of the role of genetics in schizophrenia. The need for better information about schizophrenia for the public, but also for relatives of schizophrenics, was clearly established.

PET Scan: Visualization indicated a relatively decreased frontal brain metabolism in schizophrenia.

Amphetamine-Induced Improvement: The improvement in psychosis and the relative lack of worsening during the pimozide-withdrawal phase following amphetamine infusion raised questions about generalizing the DA hypothesis of schizophrenia and suggested that DA subsensitivity may be present in chronic schizophrenia. This observation is consistent with the lack of withdrawal tardive dyskinesia following short-term neuroleptic treatment. We observed that amphetamine-induced worsening during pimozide treatment predicted early relapse after pimozide withdrawal. Lithium pretreatment decreased the activating effects of d-amphetamine but chronic pimozide pretreatment did not. Our findings support the notion of state- and time-dependent vulnerability to psychotic decompensation, which is only partially decreased with neuroleptic treatment. The d-amphetamine infusion test during neuroleptic treatment may have similar implications for neuroleptic treatment withdrawal as the dexamethasone suppression test in affective illness does for discontinuation of tricyclic antidepressants. Amphetamine-induced improvement was associated with improvement with pimozide.

Apomorphine: Blunted growth hormone response to apomorphine in poor premorbid functioning suggests a hypodopaminergic condition in these patients. This may be consistent with delayed or lack of antipsychotic effects of neuroleptics in these patients.

Decreased CSF 5HIAA in suicidal schizophrenic patients suggests that low 5HIAA levels and suicidality are not necessarily present only in depression.

Proposed Course of Project

The project will continue to investigate the effects of neuroleptic drugs, predicting drug response and the interaction of neuroleptics with biological variables and behavior such as negative symptoms. We will continue our PET and CAT scan studies, spinal fluid peptide studies and receptor function studies. The psychophysiological evaluation, in combination with biological variables, appears promising. Repeated drug-free evaluations remain an important approach in separating state from trait variables. The Section has been absorbed into the Neuroscience Branch which makes continuation of some studies uncertain.

Publications

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00081-08 BP |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 - September 30, 1982</p> | | |
| TITLE OF PROJECT (80 characters or less) Heritable Characteristics of Cation Transport in Primary Affective Disorders | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: J. I. Nurnberger, Jr., M.D. Senior Staff Fellow BP NIMH | | |
| OTHER: E. S. Gershon, M.D. Chief, Section on Psychogenetics BP NIMH | | |
| S. Simmons, M.S.N. Clinical Nurse Expert CC NURS | | |
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| D. Lawrence, B.S. Research Assistant BP NIMH | | |
| S. Pandey, Ph.D. Professor of Psychiatry Ill. State Psychiatric Institute | | |
| COOPERATING UNITS (if any) Illinois State Psychiatric Institute, Chicago, Illinois; University of Chicago | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Psychogenetics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: <p style="text-align: center;">0.5</p> | PROFESSIONAL: <p style="text-align: center;">0.2</p> | OTHER: <p style="text-align: center;">0.3</p> |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>The goal of this project is the identification of <u>heritable characteristics of ion transport</u> which may distinguish individuals with a <u>primary affective disorder</u> from normal controls.</p> <p>A study of <u>sodium potassium stimulated adenosine triphosphatase</u> ($\text{Na}^+ - \text{K}^+$-ATPase), a cation transport enzyme has been completed. Nak ATPase is a "state" but not a "trait" marker for affective illness. ATPase activity is decreased by arecoline, but this response does not differentiate patients from controls.</p> <p>We have engaged in a collaborative program of research on the <u>lithium erythrocyte/plasma ratio</u> in <u>euthymic patients</u>. We have confirmed an extended effect of lithium treatment on the ratio. Several other laboratories have asserted that lithium ratio is a genetic marker for a significant portion of bipolar patients. However, they have neglected this effect and may therefore be studying an artifact.</p> | | |

Project Description

A spectrophotometric assay was used to determine levels of $\text{Na}^+ - \text{K}^+$ - ATPase in human erythrocytes. We have previously found that NaK ATPase is a "state" but not a "trait" marker in affective disorder.

We have examined the response of the enzyme to arecoline stimulation, since arecoline has induced depressive symptoms in some subjects. Arecoline reduced NaK ATPase activity in eight patients and 13 normal volunteers. No difference was seen between the two groups. ATPase response to amphetamine with and without the blocking agents thymoxamine, propranolol and haloperidol is now being studied. We have also examined ATPase activity in red cells from 10 patients with Alzheimer's Disease and age- and sex-matched controls. No difference between groups was found. A method for determining ouabain binding in red cells and lymphocytes has also been developed.

In collaboration with Dr. Markku Linnoila of the Clinical Psychobiology Branch, 36 patients had a comparison of red cell NaK ATPase activity and plasma vanadium levels. No significant correlation was found between the two measures. This does not support theories of an etiologic role for vanadium in affective illness.

We have entered into a collaboration with Dr. G. Pandey at the Illinois State Psychiatric Institute in an attempt to replicate their finding that lithium erythrocyte/plasma ratio is a genetic marker for affective disorder. We confirmed that patients have higher ratios than controls ($p = .03$). However, if we split patients into a group off of medications 1 1/2 - 2 weeks and another group off of medications 3 weeks or more, only the first group had significantly elevated lithium ratios. Ratios in patients tested at both times tended to come back into the normal range with more time free of lithium. A number of investigators have reported that lithium ratio is a genetic vulnerability factor for many bipolar patients. We suspect these claims to be inflated by the effects of treatment.

Significance to Biomedical Research

Cation transport mechanisms depend on cell membrane characteristics that may be largely genetically determined. We have demonstrated state dependent differences in cation transport mechanisms in affectively ill patients, but have not as yet identified trait differences.

The claim that lithium erythrocyte/plasma ratio acts as a genetic vulnerability factor in pedigrees of people with affective disorder has received much attention. Our findings should provide a cautionary note to investigators in this field.

Proposed Course of Project

Analysis of the effect of amphetamine on ATPase will continue. Studies on ouabain binding and ATPase activity in patients and controls, with

identification of the subgroup of patients with especially disordered cation regulation, may continue if personnel are available.

Publications:

Nurnberger, J.I., Jr., Jimerson, D.C., Allen, J.R., Simmons, S., and Gershon, E.S.: Red cell ouabain-sensitive $\text{Na}^+\text{-K}^+\text{-ATPase}$: A state marker in affective disorder inversely related to plasma cortisol. Biol. Psychiatry, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00084-08 BP |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 - September 30, 1982</p> | | |
| TITLE OF PROJECT (80 characters or less) Genetic-Biologic Studies of Psychiatric Disorders | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
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| SECTION Section on Psychogenetics | | |
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| TOTAL MANYEARS: 7.4 | PROFESSIONAL: 6.4 | OTHER: 1.0 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Further study of <u>fibroblasts</u> demonstrated that the <u>cholinergic muscarinic receptor</u> is capable of <u>up-regulation</u>, and that cholinergic agonists inhibit <u>aderyl cyclase</u> in the fibroblasts. <u>Clinical genetic</u> study of patients and <u>ill relatives</u> reveals significantly <u>higher density of muscarinic binding sites</u> than in normal controls. Genetic control of variation in binding sites and in up-regulation has been demonstrated. Other clinical biological studies show that plasma <u>GABA</u> is significantly reduced in <u>euthymic</u> medication free patients, but platelet <u>³H-imipramine</u> binding is not different from normal controls.</p> <p>Family study data from normal controls added to data from affective patients has now shown that affective illness is highly familial, following a <u>multi-factorial (polygenic)</u> mode of inheritance in the <u>population</u>. <u>Anorexia nervosa</u> patients have high frequency of <u>bipolar</u> and <u>unipolar disorder</u> in relatives, suggesting a genetic relation between anorexia and affective disorders.</p> | | |

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| | | |
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Project Description:1. Family Study of Affective Illness

Data are now available on psychiatric illness in relatives of normal controls, chosen from the medical wards of the NIH Clinical Center, and from a population sample in New Haven, Connecticut, studied by our Yale collaborators headed by Dr. Myrna Weissman.

Considering these new data in a genetic model confirms the original analysis that multiple threshold multifactorial (polygenic) inheritance fits the distribution of illness in families and in the population. In this model, the various forms of affective disorder, Schizoaffective, Bipolar (I and II) and Unipolar illness represent progressively higher thresholds of disease manifestation on a single underlying dimension of vulnerability. Normal controls can be represented as individuals whose vulnerability score is lower than the lowest disease threshold (Unipolar).

The data collected in Bethesda (including the data on relatives of patients, included in last year's report) are as follows:

First Degree Relatives

| <u>Diagnosis of Patient</u> | <u>Diagnosis of Relatives, %</u> | | | | |
|-----------------------------|----------------------------------|-------------|--------------|-----------|----------|
| | <u>S-A</u> | <u>BP I</u> | <u>BP II</u> | <u>UP</u> | <u>N</u> |
| Schizoaffective (S-A) | 6.2 | 10.9 | 6.2 | 14.7 | 83 |
| Bipolar I (BP I) | 1.1 | 4.5 | 4.1 | 14.0 | 548 |
| Bipolar II (BP II) | 0.6 | 2.6 | 4.5 | 17.3 | 191 |
| Unipolar (UP) | 0.7 | 1.4 | 1.4 | 16.6 | 166 |
| Normal Controls | 0.5 | 0.0 | 0.5 | 5.8 | 265 |

The heritability of affective illness in the population, according to these data, is approximately 80%.

Methodological studies were performed on reliability of diagnoses derived solely from information given by relatives. For 154 persons whose direct

interview diagnosis was Unipolar, information from relatives was enough to diagnose major affective disorder in 57%. In another 31% relatives information suggested a psychiatric disorder, but the information given was not detailed enough to reach a diagnosis. In 12%, the relatives said the person was psychiatrically normal or could provide no information. We concluded that reliability of family history information was acceptable. The net amount of attenuation of observed rates of illness due to incompleteness of information from relatives is quite small, since the vast majority of individuals included in the above table were directly interviewed (in addition to having relatives give information).

Anorexia nervosa appears to be related to major affective disorders in family studies. In relatives of 24 anorectics, as compared with relatives of 44 normal controls, there is greater lifetime prevalence of major affective disorders in the families of anorectics (21.6% vs. 6.8%). There is also a slight increase in anorexia and bulimia in relatives of patients vs. relatives of controls (6.4% vs. 1.3%) which is statistically significant.

Study of children of patients with affective disorders has continued but data are not yet available.

2. Genetic Analyses of Affective Disorder in Families.

A. Relationship of HLA to major affective disorder.

A recently published report (Weitkamp et al., N. Engl. J. Med., 1981; 305:1301-6) concluded that a major susceptibility locus for depression is located in the HLA region of chromosome 6. This conclusion was based on an increased sharing of HLA haplotypes in a sub-sample of affected sib-pairs and non-random segregation of HLA types and illness in a sub-sample of families. However, the sample as a whole did not show a relationship of HLA to depression. A critique of this study was written (Goldin et al., in press) which demonstrated that: 1) there was no theoretical justification for the criteria used to select sub-samples of families; 2) the criteria led to declassification of families that was inconsistent with the stated hypothesis; and 3) their findings could not be replicated in our own data (including data from our sample that were previously unpublished).

B. Segregation analyses of affective disorder in the Amish of Pennsylvania.

A collaboration has been established with Dr. Janice Egeland who has collected data (interviews and genetic markers) from several large pedigrees ascertained through individuals with Bipolar I illness in the old order Amish community. Preliminary analyses have been carried out in an initial sample of 5 families and in a complete sample of 9 families to test single major locus hypotheses under a simple model and using a hierarchical scheme for classification of diagnoses into ill and well categories. A parallel

analysis at Yale University (Dr. D. Pauls) came up with similar results. It was found that in the initial sample of 5 families if Schizoaffective, Bipolar, Unipolar, minor depression and hypomanic disorder were classified as "ill", then a major locus hypothesis was consistent with the data and a non-genetic hypothesis was rejected. However, a dominant hypothesis with reduced penetrance could not be distinguished from a recessive hypothesis with reduced penetrance. When the entire sample of 9 families were analyzed, all genetic and non-genetic hypotheses had similar likelihoods and could not be distinguished. This inconsistency brings up the possibility of some ascertainment bias as a result of the order in which families were completed.

Other approaches will be taken to analyze these families in the future. If the families are traced back to the founding generation of some populations, some pedigrees will be more closely related than others. This information can be used to analyze families that may be homogenous for major gene segregation and linkage to genetic markers.

3. Evaluation of Adoption Study Methodology.

We have reviewed strategies of adoption studies in behavioral disorders in order to evaluate the biases in various designs. The least biased design is the comparison of prevalence of an illness in adoptees according to the illness status of their biological and adoptive parents (cross-fostering design). We also raised two issues which may always be a problem in adoption studies. One problem in adoption studies is possible matching of adoptive parents to biological parents for some trait which is correlated to the illness in question. We have examined this problem quantitatively and have determined that if an illness is strictly environmental and there is a sub-group in the population with a relatively "high" prevalence of an illness (while the remainder of the population has a "low" prevalence) and there is complete matching of biological and adoptive parents by sub-group, then this can simulate a genetic effect. That is, a significantly higher proportion of adoptees will be affected if their biological parent is affected than if their biological parent is unaffected. While this does not negate findings in the literature, it raises the issue of whether or not adoption studies can actually separate genetic and environmental components.

4. Optimal Strategies for Demonstration of Genetic Linkage Between a Disease and a Specific DNA Region.

A new area of research in psychobiology in which we are involved is the search for a restriction enzyme polymorphism in a specific region of DNA that is linked to a gene causing susceptibility for a particular psychiatric disorder.

Because this research is cumbersome, we are now doing theoretical calculations to determine the optimal strategy for performing these experiments. We are attempting to determine: 1) how many restriction enzymes should be tested; 2) what type of data to collect (i.e., nuclear families, pedigrees, affected sib-pairs); 3) how many individuals or families to study.

In order to answer these questions we need to know the expected frequency of restriction enzyme polymorphisms. Because this may not be known, we may have to assume some range of reasonable values. We also need to consider the relative ease of sampling certain numbers of relatives. That is, it may be easier to obtain samples from affected sib-pairs than from entire families.

5. Molecular Genetic Studies of Psycho- and Neurobiological Problems.

A molecular genetics laboratory using recently developed DNA technologies has been set up in this Section.

The projects currently in progress in the molecular genetics laboratory are two-faceted, i.e., clinical and basic. Investigations concerning identification of possible DNA restriction length polymorphisms in psychiatric patients constitutes the clinical project. We are studying the gene for pro-opiomelanocortin, the precursor protein of several psychobiologically important peptides, including ACTH and beta endorphin. The prospective polymorphic site(s) could eventually serve as genetic markers of vulnerability to altered hormonal or psychiatric function. The experiments under progress related to this study include preparations of DNA from about 30 patients, selective restriction enzyme digestions and blot hybridizations of the purified DNA samples using a radiolabelled human DNA fragment containing the gene cluster for pro-opiomelanocortin.

The second study concerns cloning DNA fragments encoding other proteins of neurobiologic significance, e.g., beta-adrenergic receptor. As a first step towards this goal, efforts are concentrated on constructing clone libraries of complementary DNA synthesized off total messenger RNA from the sources supposed to be rich in the appropriate message; HeLa cell line and rat brain are two such sources presently being explored.

6. Cellular Models of Neuronal Function.

We have set up a laboratory dedicated to the culturing and biochemical study of the fibroblasts obtained from patients with manic-depressive illness, their ill siblings and normal volunteers who have been screened in our clinic and ascertained to be free of psychiatric illness. These cell lines serve as possible models of cell membrane receptors and other processes in the nervous system, and allow direct study of genetic variation among individuals. We investigated the expression of gene coding for neurotransmitter receptors on the fibroblast membranes. Our findings confirmed the existence of a beta-receptor in the fibroblast membranes. In addition, we have demonstrated the existence of a muscarinic cholinergic receptor in the cultured fibroblast. Other receptors which were screened for included alpha-bungarotoxin, angiotensin, vasopressin, dihydromorphine, thyrotropin releasing hormone, GABA, serotonin, dopamine and diazepam. With the exception of dopamine, serotonin and diazepam, our findings were all negative, i.e., the total binding of the labelled ligand was not different from the nonspecific binding. In the case of dopamine, binding did occur but it did not exhibit stereospecificity. In the case of serotonin, there was binding but no saturability was observed. With diazepam a "saturable" binding site was observed but it

was of the peripheral binding type, i.e., it had the same characteristics as diazepam binding to kidney as opposed to brain. Current plans include the investigation of the existence of other peptide receptors such as the enkephalin receptor in fibroblast lines.

In view of the theory on cholinergic hypersensitivity advanced by Janowski et al. and supported by sleep studies in our laboratories, the discovery of the muscarinic cholinergic receptor (hereinafter referred to as the QNB receptor, after the labelled ligand used to measure it) was studied in detail over the course of the year. The first studies involved determining how similar the peripheral QNB receptor was to the central nervous system (CNS) one. This was carried out by studying the effect of drugs to displace QNB binding in human fibroblast and rat brain membranes:

| $10^{-5}M$ drug | Fibroblasts | CNS |
|---------------------|----------------|----------------|
| carbamyl choline | 68.2 ± 0.7 | 35.2 ± 6.0 |
| oxotremonine | 43.1 ± 2.1 | 70.1 ± 6.0 |
| scopolamine | 100 ± 1.5 | 100 ± 0.6 |
| atropine | 100 ± 4.8 | 100 ± 4 |
| dopamine | 69 ± 0.7 | 58 ± 5 |
| 5-hydroxytryptamine | 25 ± 0.8 | 21 ± 2 |

The intraclass correlation coefficient is high ($r = 0.88$, $p < 0.01$), indicating that structurally these two sites are very similar indeed. Furthermore, like brain receptors, exposure to atropine increases the number of QNB receptors reinforcing the similarity concept. In addition, the several different cell lines investigated show that there are stable differences between lines in the inductions of receptor sites. This observation is suggestive of genetic control of the QNB binding sites. The brain receptor is also known to down-regulate in response to cholinergic agonists such as arecoline and oxotremonine. We have failed to find down regulation in our fibroblast system; the reasons for it are currently being investigated.

Having obtained good evidence for similarities between the brain and the peripheral receptor, we then attempted to see if the QNB binding in fibroblasts from patients and their ill siblings was different from QNB binding in fibroblasts from normal volunteers. Preliminary evidence shown indicates that the number of QNB binding sites in normal fibroblasts is lower than in the patients ($p < .001$), with no difference in K_D . The ill relatives of these patients also have increased QNB binding ($p < .002$).

In addition to studying the surface receptor, we have been looking at the second message systems in the fibroblasts. We have so far demonstrated that cholinergic agonists inhibit the adenylyl cyclase system in fibroblasts. The investigation of any differences between this system in patients and controls is under current investigation. Studies of calmodulin mediated membrane protein phosphorylation are also being initiated at this time in collaboration with Dr. P. Marangos.

In yet another aspect of the biochemical investigation of the fibroblasts we have studied the high affinity uptake of choline. We found no

difference in this parameter between patients and normal volunteers, 6.7 ± 0.5 pmol/min/mg protein (N = 18), 5.9 ± 0.7 pmol/min/mg protein (N = 5), respectively. As a tangent to this study we have also undertaken the study of choline uptake in fibroblasts from patients with Alzheimer's Disease as compared to age- and sex-matched controls. In this case we found a difference between controls and patients of 6.1 ± 0.6 pmol/min/mg protein (N = 5) vs. 4.4 ± 0.4 (N = 4) pmol/min/mg protein ($p < 0.001$ student's t-test).

Future projects in the fibroblast laboratory involve the establishing of "libraries" of fibroblasts from multigenerational pedigrees in which both ill and well relatives may be obtained. The analysis of such a system may give some further insight into the transmission of the defective expression of a receptor. The fibroblasts which are currently in the laboratory and the future ones will be used in the determination of whether they express neural peptides such as endorphins, thyrotropin releasing hormone, etc.

Lymphocytes from patients with manic depressive illness and normal volunteers have also been studied in our laboratory for a number of receptors. We have confirmed the existence of QNB binding and beta-adrenergic binding on these cells. We have been unable to replicate the binding of dopamine to these cells. Studies are currently being conducted to determine the reason for this discrepancy. We have used the QNB binding on the lymphocytes to distinguish between patients and normal volunteers. The studies to date indicate that in the euthymic patients (the majority of patients seen in our clinic) QNB binding does allow a distinction between patients and controls.

In collaboration with Dr. Pat Randels of the Coatesville VA Hospital, Coatesville, Pennsylvania, we studied 10 unmedicated male patients with Alzheimer's Disease and 10 age-matched male controls. We measured platelet MAO, platelet vasopressin receptor, platelet GABA-transaminase, plasma vasopressin, plasma GABA, serum cortisol, lymphocyte acetylcholine receptors, erythrocyte choline uptake, erythrocyte $\text{Na}^+\text{-K}^+$ ATPase and erythrocyte ouabain binding. Of these variables, mean serum cortisol was significantly higher among the patients and erythrocyte choline uptake was significantly lower. It is planned that we will expand this collaboration, obtaining skin biopsies from these patients to measure fibroblast choline uptake.

7. Clinical and Animal Biologic Studies.

We have been studying serum and urinary cortisol from 24 patients and 14 normal volunteers in our clinic. The aim has been to demonstrate whether the hypersecretion of cortisol is a state or trait marker. The results at this point are that a) there is no correlation between urine and serum free cortisol, and b) no overall difference in serum or urinary cortisol between Bipolar patients and controls. Interestingly, in a recent study of Cushing's patients who belonged to a subgroup which, in addition to demonstrating the adrenal-pituitary axis malfunction, had depression, we were able to show that a high blood cortisol level was accompanied by increased QNB binding sites in the lymphocytes.

In an attempt to link the effect of cortisol levels and QNB binding in brain we have been studying the effect of adrenalectomy on muscarinic binding in the rat brain. At this stage we have established that following adrenalectomy there is a 50% drop in the number of QNB receptors in the rat olfactory bulb, but this number is restored to normal values following treatment with dexamethasone. Studies are under way to determine if exposure to dexamethasone with no surgery will increase the binding levels of QNB, and also to use hypophysectomized rats to determine the relationship between ACTH levels, corticosterone levels and QNB binding in the brain. We are also determining whether the adrenalectomy effect is specific. To date our studies have failed to show any difference in beta-binding. Currently we are investigating the effect of adrenalectomy on other ligands and other sites of the rat limbic system. Establishing a correlation between the levels of corticosterone in the QNB binding in the brain of the rat may help better understand the patients with pituitary-adrenal axis disturbance and cholinergic receptor supersensitivity.

³H-imipramine binding sites in brain and in platelets have been linked to serotonin uptake systems, making these binding sites of interest in the study of affective disorders because of an extensive body of literature relating altered serotonergic function to affective disorders.

The number of ³H-imipramine binding sites in platelets has been reported to be decreased in depressed patients compared to normal volunteers. To determine whether this biochemical difference would be useful as a possible marker for genetic vulnerability to affective disorders, we studied ³H-imipramine binding in 12 unmedicated euthymic Bipolar patients and 12 matched controls. There were no differences between the two groups in either number of binding sites (B_{max}) or affinity (K_d). This suggests that the reported decrease may not be a state-independent marker. However, given that the reported difference between patient and control groups is approximately 1 standard deviation of the mean, the risk of a Type II error in a sample size of 24 is approximately 25%.

Several parameters of GABAergic physiology have been studied in euthymic Bipolar patients to determine whether these parameters could be markers for genetic vulnerability to affective disorders. We have established that platelet GABA-transaminase (GABA-T) and plasma GABA are concordant in monozygotic twins. We have found that platelet GABA-T and CSF and plasma GABA are significantly lower in euthymic, unmedicated Bipolar patients. Interestingly, lithium treatment elevates both plasma GABA ($p < .02$) and CSF GABA ($p < .02$) levels.

Currently, we are expanding these studies to include other parameters of GABAergic physiology, such as platelet GABA uptake and serum and CSF glutamic acid decarboxylase. We plan to study these GABAergic parameters in unmedicated euthymic Bipolar patients and in lithium-treated euthymic Bipolar patients to determine the mechanism underlying the lithium-induced increases in CSF and plasma GABA. Preliminary results indicate platelets from 20 lithium-treated euthymic Bipolar patients have decreased GABA uptake ($p < .01$) compared to 20 normal volunteer.

Additionally, we have an approved protocol for conducting GABA infusion experiments with normal volunteers and unmedicated Bipolar patients. We plan to give 1 mg/kg doses, examining GABA kinetics, mood responses and neuroendocrine effects.

Recently, Gold et al. (Lancet 1: 1233, 1978) hypothesized that central vasopressin function may be diminished in depressed patients on the basis of several lines of evidence including their finding that CSF vasopressin was lower in non-psychotically depressed patients. We measured vasopressin platelet receptor function in euthymic medication-free Bipolar patients, in lithium-treated euthymic Bipolar patients and in normal volunteers to assess in a preliminary fashion this aspect of vasopressin function in affective disorders. There were no differences in either affinity or number of binding sites among these three groups. We will attempt to measure these parameters in unmedicated depressed patients as part of our effort to evaluate vasopressin in affective disorders.

Significance to Biomedical Research and the Program of the Institute

Successful identification of a marker of genetic vulnerability to affective disorders would lead to identification of the responsible pathophysiological process, and would have clinical applications for prevention and choice of treatment. Identification of a muscarinic receptor on fibroblasts, and demonstration of higher receptor density in patients with affective illness, are steps accomplished this year. The cholinergic receptor on the fibroblast may prove to be a valid genetic marker which would lend support to a cholinergic hypothesis in affective disorders.

Diagnostic genetic studies, including methodologic studies, contribute to delineation of a set of clinical syndromes inherited together. The acceptable fit of polygenic (multifactorial) inheritance models to affective illness implies that Schizoaffective disorder, Bipolar illness and Unipolar illness share a common vulnerability factor which is most strongly present in Schizoaffective illness and least strongly in Unipolar illness. Anorexia nervosa appears to share the same vulnerability factor. This vulnerability factor can be sought in future biological studies.

The systematic study of ³H-imipramine binding, plasma and CSF GABA, the relation of corticosteroids to cholinergic function, and lithium membrane transport will allow us to state whether they are implicated in genetic vulnerability to affective illness.

The DNA laboratory is the first of its kind in clinical genetic investigation in psychiatry, and its initial project will allow us to demonstrate whether a structural variation in the gene for pro-opiomelanocortin, whose biological derivatives include ACTH or beta-endorphin, is implicated in affective illness or Cushing's Disease.

Proposed Course of Project

We plan to continue to investigate the biology and genetics of characteristics that may be implicated in the genetics of affective disorders,

as described above. Establishing a library of DNA and living cells from entire pedigrees is a major priority; our family study resources recently have been in the direction from collection of clinical diagnostic data. Mathematical methodology for clinical investigation will continue to be studied.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00085-06 BP |
| PERIOD COVERED October 1, 1981 - September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Pharmacogenetics of Psychoactive Drugs | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: J. I. Nurnberger, Jr., M.D. Senior Staff Fellow BP NIMH | | |
| OTHER: E. S. Gershon, M.D. Chief, Section on Psychogenetics BP NIMH S. Simmons, M.S.N. Clinical Nurse Expert CC NURS J. Guroff, B.A. Research Assistant BP NIMH D. C. Jimerson, M.D. Staff Psychiatrist BP NIMH S. Nadi, Ph.D. Research Biochemist BP NIMH J. Schreiber, M.S.W. Social Worker BP NIMH D. Goldstein, M.D. Senior Staff Fellow HE NIHLB H. Keiser, M.D. Clinical Director OD NIHLB | | |
| COOPERATING UNITS (if any) Laboratory of Clinical Science and Clinical Psychobiology Branch, NIMH; Clinical Center Nursing Department, NIH; Food and Drug Administration; Univer- sity of Maryland; NHBLI | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Psychogenetics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.1 | PROFESSIONAL: 0.8 | OTHER: 0.3 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Behavioral and biological responses to the muscarinic cholinergic agonist</u> <u>arecoline were studied in bipolar patients and normal twins. Several</u> <u>behavioral response parameters and the prolactin response were concordant</u> <u>in monozygotic twins, suggesting a heritable component. Behavioral, neuro-</u> <u>endocrine, and psychophysiologic responses to arecoline did not distinguish</u> <u>the euthymic patients from controls however.</u> <u>Amphetamine responses in normal volunteers have been further characterized</u> <u>by the use of the blocking agents propranolol, thymoxamine and haloperidol.</u> Data is now being analyzed. | | |

Other Investigators Continued:

| | | | |
|-----------------------|--------------------|----|------|
| D.C. Jimerson, M.D. | Staff Psychiatrist | BP | NIMH |
| S.S. Jimerson, M.S.N. | Guest Worker | BP | NIMH |
| L. Kessler, M.D. | Guest Worker | BP | NIMH |

Project Description:

Pharmacogenetic studies in psychopharmacology have largely been devoted to inherited differences in drug metabolism as opposed to genetics of physiologic and psychologic responses to drugs. The behavioral pharmacogenetics of psychoactive drugs in particular is an area in which little systematic investigation has been undertaken.

Cholinergic supersensitivity has been postulated to be an etiologic factor in affective disorder. We administered 8 mgm of the muscarinic agonist arecoline subcutaneously to 8 pairs of normal volunteer identical twins and 8 bipolar patients currently euthymic and off medications. During the hour following arecoline, the Profile of Mood States (POMS) showed an increase in total mood disturbance in both patient and control populations. Mean systolic blood pressure, pulse, plasma cortisol, prolactin, and growth hormone also increased. Baseline cholinergic receptor binding was measured in lymphocytes but did not predict behavioral or hormonal response to arecoline. Anger and elation scores on the POMS showed significant concordance in identical twins, as did change in prolactin, implying that these are the components of drug response most likely to be influenced by genetic factors. None of the responses differentiated well state patients from controls. We were thus unable to demonstrate cholinergic supersensitivity in the regulation of mood, neuro-endocrine, or autonomic function in bipolar affective illness.

We previously reported that the behavioral excitation response to amphetamine, as well as the prolactin and growth hormone responses, were highly concordant in monozygotic twins. In an effort to neurochemically characterize these and other amphetamine responses, we administered the blocking agents propranolol, thymoxamine, and haloperidol on separate days to normal volunteers prior to giving an amphetamine infusion (0.3 mg/kg over 5 minutes). Ten volunteers received haloperidol, 9 received propranolol and 8 received thymoxamine. Preliminary results indicate that thymoxamine attenuates the cortisol response to amphetamine.

In the course of the above study blood pressure response to amphetamine has been found to be highly correlated with plasma norepinephrine response. This collaborative study with Dr. D. Goldstein and Dr. H. Keiser of NHBLI has led to interest in using amphetamine or tyramine in a study of persons at risk for hypertension.

A twin study of the cholinergic REM induction response has been completed. Seven monozygotic twin pairs were tested. All of these twins had responded

to a call for normal volunteers. However, three of the twin pairs included one member with a history of minor affective illness and one additional twin who had suffered an episode of major affective disorder. All twins were euthymic at the time of testing. A statistically significant concordance in REM induction time was noted (intraclass correlation = 0.69, $F = 5.35$, $p = .02$), while REM latency following placebo was not significantly concordant. No association between REM induction time and affective illness was found in this group which leads us to conclude that cholinergic supersensitivity may predominantly be present only in groups of severely ill affective patients. The twin study does suggest a possible heritable variation in central muscarinic receptor sensitivity.

Proposed Course of Project:

Pedigree studies of REM induction by arecoline will be performed in families of patients with affective illness. Cholinergic sensitivity is also being tested in an *in vitro* system using cultured fibroblasts (Z01 MH 00084-08 BP). We have demonstrated an abnormality in the GABA system in affective disorder (see Z01 MH 00084-08 BP) and are planning a study of responses to intravenous GABA infusions in patients and controls.

Significance to Biomedical Research:

The cholinergic system has received much attention recently in affective illness. Our study has demonstrated that the supersensitive response we previously reported (the cholinergic REM induction test) does not imply a generalized cholinergic receptor supersensitivity in bipolar illness.

The twin study of the cholinergic REM induction response provides further evidence that this response relates to heritable variation in muscarinic receptor sensitivity in man. This response may be a marker for genetic susceptibility to affective illness. The blockade study of amphetamine responses will enable us to characterize the heritable variation in behavioral and neuroendocrine measures we observed in our original normal twin study. This has implications for understanding genetically-mediated variation in human brain function.

Publications:

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00086-06 BP |
| PERIOD COVERED October 1, 1981 - September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: E. S. Gershon, M.D. Chief, Section on Psychogenetics BP NIMH | | |
| OTHER: J. I. Nurnberger, Jr., M.D. Senior Staff Fellow BP NIMH S. Simmons, M.S.N. Clinical Nurse Expert CC NURS D. C. Jimerson, M.D. Staff Psychiatrist BP NIMH W. Berrettini, M.D., Ph.D. Staff Psychiatrist BP NIMH M. Kafka, Ph.D. Research Associate BP NIMH L. Kessler, M.D. Guest Worker BP NIMH A. Lewy, M.D. Staff Psychiatrist BP NIMH W. Potter, M.D. Acting Chief CP NIMH D. Selinger, M.D. Psychiatrist Hadassah Univ. Hospital | | |
| COOPERATING UNITS (if any) Clinical Center Nursing Department, NIH; University of Oregon, Food and Drug Administration; Hadassah University Hospital | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Psychogenetics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 3.5 PROFESSIONAL: 2.7 OTHER: 0.8 | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>The outpatient clinic provides a "well state" group of 90 patients for <u>genetic and pharmacologic studies of persons with Bipolar (manic-depressive) disorders and Unipolar (major depressive) disorders</u>. The clinic also studies treatment response to medications, particularly in Bipolar disorder.</p> <p>There are 23 <u>normal twin pairs</u> and 23 <u>normal volunteers</u> who serve as controls for these studies.</p> <p>We have developed the use of an assay for <u>lithium</u> in <u>parotid saliva</u> as a clinical alternative to blood lithium levels.</p> <p>Eleven Bipolar patients have participated in our study of <u>trihexyphenidyl</u> as an antidepressant. Preliminary results are promising. Three patients have participated in the antidepressant study of <u>pirbuterol</u>.</p> | | |

Project Description:

The purpose of the project is to have a "well state" patient population and comparison groups available for participation in the following research areas:

1. Identification of biochemical, pharmacologic, or physiologic abnormalities which are present in persons who have had major affective disorder but who do not currently require hospitalization.
2. Identification of biological markers of a genetically transmitted predisposition to affective disorders.
3. Creation of a pool of available patients and relatives for further protocols which will be developed and submitted on the genetics of affective disorders, and on longitudinal studies of affective disorders.
4. We presently maintain an active roster of 23 pairs of normal monozygotic twins and 23 singletons for participation in genetic biological studies (Z01 MH 00084-08) and pharmacogenetic studies (Z01 MH 00085-06 BP)

Patients are admitted to the study upon referral from the wards of the NIMH Intramural Program or from various community referral resources. Ninety patients are currently enrolled in the BPB portion of the outpatient clinic (75 Bipolar, 11 Unipolar and 4 Schizoaffective). Twins and singletons are recruited through the normal volunteer office or by advertisement.

Identification of biochemical, pharmacologic or physiologic vulnerabilities to affective illness: The strategy in this type of study is to identify a biologic vulnerability to illness which is not state dependent but present or evocable even when the patient is in the "well state" or in remission.

We have described the results of our twin and patient studies of behavioral, hormonal and sleep responses to the muscarinic agonist arecoline (Z01 MH00085-06 BP). The cholinergic REM induction test may be useful as a trait marker in affective disorder.

Preliminary data from our double-blind, placebo-controlled eight week trial of trihexyphenidyl (Artane) as an antidepressant have been analyzed. This trial is the first such controlled trial conducted and is of significant theoretical interest because of prominent cholinergic theories of the defect in affective disorder. Bipolar patients have been continued on lithium throughout the trial whether or not they receive active Artane. Eleven patients have started the drug trial so far. Three patients dropped out before four weeks: one with increasing depression, one with nausea (but improved mood) and one with improved mood who was noncompliant in keeping appointments. One patient was dropped from the protocol due to alcohol abuse and one patient is presently in the middle of the trial. Six patients have completed the trial, two on placebo and four on active Artane. Of the patients on placebo,

one had increasing depression and one became hypomanic. Of the patients on Artane, two had fairly dramatic antidepressant responses and two had moderate responses. Three out of four were judged to have significant clinical improvement at the end of the eight weeks, and therefore continued on Artane on an open basis. Two of these patients have now been maintained on lithium and Artane for over a year with no episodes of major mood disorder.

In the trial of the beta agonist pirbuterol, three patients have participated. One patient dropped out in the second week with increasing depressive symptoms. Two patients finished the trial on active pirbuterol; both of these patients experienced moderate improvement. Neither was felt to have sufficient clinical benefit to continue the drug on an open basis.

It is often difficult to monitor serum lithium levels in patients with poor veins or in children. Previous methods of determining salivary lithium concentrations have been unreliable. In collaboration with Dr. Drora Selinger, a guest worker, we have developed a simple method for obtaining reliable salivary lithium levels. Using a suction cup attached to the opening of the parotid gland on the inside of the cheek, and stimulating salivary flow with a weak citric acid solution, we obtained salivary samples from seven patients on lithium carbonate treatment. The correlation between salivary and serum lithium levels was 0.98 ($p < .001$). In a further study, we demonstrated that this measurement could be done despite the presence of anticholinergic drugs.

A new clinical protocol involves study of CSF in euthymic bipolar outpatients. This is the first lumbar puncture protocol at NIMH to be conducted with outpatients. Preliminary evidence suggests that CSF GABA, reported to be lower in the depressed state in Bipolar patients, is also lower in the euthymic state. Lithium treatment elevates the CSF GABA level. To date, we have performed lumbar punctures on 12 Bipolar subjects and on 5 normal volunteers.

Significance of the Study:

The genetic-biochemical studies in our clinic population are part of a search for biologic markers of genetic vulnerability to affective illness, as detailed in our other project reports.

Our antidepressant studies have two goals: 1) further understanding of the neurochemical etiology of affective illness; and 2) better clinical treatments for bipolar disorder. Initial results provide confirmatory evidence for the cholinergic hypothesis of mood disorders.

The salivary studies provide a new clinical tool in the monitoring of patients on lithium.

Proposed Course of Study:

We expect a continuation of the gradual growth of our clinic population. We are now limiting entrance to patients with bipolar or episodic schizoaffective illness and are concentrating on patients who take lithium alone and who may be taken off all medications for several weeks at a time for biologic and pharmacologic studies.

Our antidepressant studies will continue through the coming year, as will the CSF study.

Publications:

Targum, S.D. and Gershon, E.S.: Pregnancy, genetic counseling and the major psychiatric disorders. In Schulman, J. and Simpson, J.L. (Eds.): Genetic Diseases and Pregnancy. New York, N.Y., Academic Press, 1981, pp. 413-438.

Frank, E., Targum, S.D., Gershon, E.S., Anderson, C., Stewart, B.D., Davenport, Y., Ketchum, K.L., and Kupfer, D.J.: A comparison of non-patient and Bipolar patient-well spouse couples. Am. J. Psychiatry 138: 764-768, 1981.

Targum, S.D., Dibble, E.D., Davenport, Y.B., and Gershon, E.S.: The family attitudes questionnaire: patients' and spouses' views of Bipolar illness. Arch. Gen. Psychiatry 38: 562-568, 1981.

Selinger, D., Hailer, A.W., Nurnberger, J.I., Jr., Simmons, S., and Gershon, E.S.: A new method for the use of salivary lithium concentrations as an indicator of plasma lithium levels. Biol. Psychiatry 17: 99-102, 1982.

Connelly, C.E., Davenport, Y.D., and Nurnberger, J.I., Jr.: Treatment compliance in a lithium clinic population. Arch. Gen. Psychiatry 39: 585-588, 1982.

Selinger, D., Simmons, S., Hailer, A.W., Nurnberger, J.I., Jr., and Gershon, E.S.: An effective method for measuring salivary lithium in patients on anticholinergic drugs. J. Affective Disord., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00117-07 BP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) α -Adrenergic and Prostaglandin Receptors in Human Blood Elements | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> PI: Marian S. Kafka Physiologist BP NIMH </div> | | |
| COOPERATING UNITS (if any) Section on Psychobiology, BPB, NIMH; Section on Neuropsychopharmacology, BPB, NIMH; Clinical Psychobiology Branch, NIMH | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Biochemistry and Pharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 0.4 | PROFESSIONAL: 0.4 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>α-adrenergic receptors and prostaglandin receptors</u> are being studied in <u>human blood cell preparations</u> . The ability of adrenergic agonists to inhibit <u>adenylate cyclase</u> is used as a measure of the receptors' biological function. Binding of tritiated adrenergic receptor antagonists to membrane receptors on platelets is being measured. These correlative measures are used to assess α -adrenergic physiological function in normal human beings, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents. | | |

Objectives:

- (1) To study alterations in receptor number or function in pathological conditions characterized by altered receptor sensitivity and in psychiatric diseases.
- (2) To provide an experimental model for the study of drug and/or physiologically-induced changes in central receptor sensitivity in man.
- (3) To understand the membrane perturbations or alterations connecting receptors, occupied by their agonists, with the inhibition of adenylate cyclase and subsequent physiological events.

Methods Employed:

To measure α -adrenergic receptors, human platelets are prepared from a fresh blood sample. A portion of the platelets is washed and resuspended for measurement of cyclic AMP production. A second portion is homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α -adrenergic antagonist binding.

Major Findings:

- (1) Platelets from young male controls make less cyclic AMP than platelets from adult male controls and make amounts similar to those in platelets from adult women.
- (2) Platelets from schizophrenics make less cyclic AMP than platelets from control subjects.
- (3) Platelets from patients with essential hypertension make less cyclic AMP than platelets from control subjects.
- (4) The number of α -receptors is greater in platelets from schizophrenic than in platelets from control subjects.
- (5) The number of α -receptors is greater in platelets from patients with affective disorders.
- (6) Platelets from patients with Parkinson's Disease and control subjects have similar numbers of α -receptors and cAMP production.
- (7) Long-term administration of neuroleptics or lithium carbonate to schizophrenic patients does not change the number of α -receptors nor cAMP production. Chronic administration of high doses of propranolol increases PGE₁-stimulated cAMP production.

Significance to Biomedical Research and to the Program of the Institute:

The results obtained from these experiments are directly applicable to an assessment of receptor function in human disease states and in monitoring the physiological effects of various drug treatments. If the peripheral

α -adrenergic receptors are related to, or can serve as models for, central nervous system adrenergic receptors, valuable information may be obtained about the functioning of these central receptors in psychiatric diseases.

It is possible that adrenergic receptor function in the central and peripheral nervous systems is similar to that measured in platelets. Platelets from schizophrenic patients make less cyclic AMP. The decrease in cyclic AMP production is due, at least in part, to the decrease in the activity of the platelet adenylate cyclase activity. If cyclic AMP production is decreased in neurons in the central nervous system of schizophrenic patients, there may be a decrease in phosphorylation and activation of some cell proteins, resulting in the deficits that characterize schizophrenia. If the number of α -adrenergic receptors is higher in the neurons, as well as in the platelets, of schizophrenic and affectively ill patients, transmission in the central nervous system may be altered: norepinephrine or epinephrine occupancy of increased numbers of pre- and post-synaptic α -adrenergic receptors might inhibit brain inhibitory centers, increasing excitation in parts of the brain under tonic inhibitory control. The excitation might contribute to the symptomatology and course of schizophrenia and affective illness.

As platelets from patients with Parkinson's disease have no alteration in receptor number or cAMP production, it is probable that chronicity or hospitalization as such are not responsible for the platelet changes observed in the other diseases. As the platelets of boys make less cAMP than those of men and amounts similar to those of women, it is possible that hormones may modulate cAMP production in the platelet (and perhaps the neuron).

Proposed Course:

Examination of the mechanism of information transfer from α -adrenergic receptors to adenylate cyclase is continuing. Studies of these receptors in patients with hypotension, as well as further studies of α -receptor function in schizophrenia and affective illness are under way.

Publications:

1. Kafka, M.S., van Kammen, D.P., Kleinman, J.E., Nurnberger, J.I., Siever, L.J., Uhde, T.W., and Polinsky, R.J.: Alpha-adrenergic receptor function in schizophrenia, affective disorder, and some diseases of neural transmission. In Jansson, B., Perris, C., and Strewé, G., (Eds.): Proceedings of the III World Congress of Biological Psychiatry. Amsterdam, Elsevier/North Holland Press, 1982, pp. 691-694.
2. Kafka, M.S. and van Kammen, D.P.: Alpha-adrenergic receptor function in schizophrenia: receptor number, cyclic AMP production, adenylate cyclase activity and the effect of drugs. Arch. Gen. Psychiatry, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <p style="text-align: center;">201 MH 00124-05 BP</p> |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 through September 30, 1982</p> | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Mechanism of Action of Lithium in the Treatment of Affective Disorders</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| P.I. Agu Pert, Ph.D. Candace Pert, Ph.D. William E. Bunney, Jr., M.D. Robert Hruska, M.D. | Psychologist Pharmacologist Staff Fellow | BP NIMH NB NIMH BP NIMH OD NINCDS |
| COOPERATING UNITS (if any) <p style="text-align: center;">Office of the Director, NINCDS</p> | | |
| LAB/BRANCH <p style="text-align: center;">Biological Psychiatry Branch</p> | | |
| SECTION <p style="text-align: center;">Section on Biochemistry and Pharmacology</p> | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | |
| TOTAL MANYEARS: <p style="text-align: center;">2.0</p> | PROFESSIONAL: <p style="text-align: center;">1.0</p> | OTHER: <p style="text-align: center;">1.0</p> |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input checked="" type="checkbox"/> (c) NEITHER </div> </div> | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> Chronic treatment of rats with <u>lithium</u> for three weeks decreased the binding of <u>[³H]-spiroperidol</u> to the <u>caudate nucleus</u> as revealed by <u>autoradiographic</u> techniques. Similar treatment also decreased <u>opiate receptors</u> in this structure. The <u>in vitro</u> addition of <u>lithium</u> to tissue homogenates from the striatum, cortex or hippocampus was found to inhibit the binding of [³H]-<u>QNB</u>. However, this was not due to an alteration in <u>muscarinic cholinergic receptors</u> since washing the tissue to remove the lithium reversed the inhibition. Chronic <u>exposure</u> of rats to lithium also did not produce any permanent changes in density of muscarinic binding sites. </p> | | |

Objectives

It has recently been suggested that oscillations in catecholamine receptor sensitivity may underlie the etiology of affective disorders, especially manic-depressive illness. We have previously found that chronic lithium treatment appeared to prevent the development of dopamine receptor supersensitivity. These findings were taken to suggest that lithium may produce its therapeutic effects by dampening the oscillations in dopamine receptor sensitivity. Subsequent to these initial findings, we also demonstrated similar effects for the alpha- and beta-adrenergic receptor systems. This series of studies was designed to further extend the analysis of the effects of lithium on neurotransmitter receptor sensitivity.

Autoradiographic Analysis of Dopamine and Opiate Receptor Binding in the Rat Brain Following Chronic Lithium

Acute lithium has no effect on the binding of [3]-spiroperidol when it is added to rat brain homogenates. We also failed to detect any effects of chronic lithium on the binding of [3 H]-spiroperidol one week following the termination of chronic lithium treatment. In this study we examined the effects of chronic lithium on the binding of [3 H]-spiroperidol and [3 H]-dihydromorphine in rat caudate using autoradiographic procedures. Rats were treated for three weeks with a lithium diet which reduced plasma lithium levels equivalent to therapeutic levels in humans. Following three weeks of treatment, the rats were killed by decapitation and their brains were removed. Sections of striatum were prepared and incubated with [3 H]-DHM and [3 H]-spiroperidol. The sections were then subjected to autoradiography. Control brains were compared to lithium-treated brains using grain count analysis or computer optical density analysis.

Effect of Chronic Lithium on the Muscarinic Cholinergic Receptor

Behavioral data from our laboratory has suggested that there is an interactive effect between chronic lithium and cholinomimetic compounds. In this series of studies we evaluated the effects of chronic lithium on the binding of [3 H]-QNB, a muscarinic cholinergic receptor ligand, in various regions of the brain following either chronic or acute in vitro lithium treatment. Rats were treated for three weeks with a lithium diet designed to induce lithium plasma levels of 0.7-0.9 mEq/ liter. After three weeks, the rats were sacrificed and the brains removed and frozen. The septum, hippocampus, cortex and striatum were dissected out and assayed for [3 H]-QNB binding under various conditions. [3 H]-QNB binding to homogenates from these brain regions was also evaluated following the addition of various concentrations of lithium to untreated rats.

Major Findings

We have previously reported that chronic lithium produces a decrease in dopamine receptors in brain homogenates from the caudate nucleus. After one week of chronic lithium treatment, there is a small but non-significant reduction in [3 H]-spiroperidol binding in the caudate nucleus. After two and three weeks, [3 H]-spiroperidol binding decreased 26.3% and 26.9%, respectively, from the control. Autoradiographic analysis also revealed a similar pattern. Dopamine recep-

tors in the caudate nucleus were decreased considerably following three weeks of treatment. Opiate receptors in this region were also affected in the same manner by chronic lithium treatment. Lithium also appears to have influences on the muscarinic cholinergic system. The in vitro addition of lithium was found to inhibit the binding of [³H]-QNB to tissue homogenates prepared from the striatum, cortex or hippocampus. This in vitro inhibition of binding was reversible, since washing the tissue to remove the lithium removed the inhibition. Chronic exposure of rats to lithium did not alter the density of muscarinic receptors. While chronic lithium does not alter receptor number, it does appear to interfere with muscarinic cholinergic receptors in vivo. This effect of lithium should be considered in evaluating its pharmacological spectrum of actions.

Significance to Biomedical Research and the Program of the Institute

Lithium is one of the most widely used and efficacious compounds in the treatment of mania and manic-depressive illness. Understanding its mechanisms of action will aid in defining the factors underlying the expression of mania and manic-depressive illness and in developing new strategies in dealing with these disorders.

Proposed Course

1. The effects of chronic lithium will be evaluated on several different receptor systems using autoradiographic procedures.
2. An effort will be made to evaluate the effects of lithium on several populations of dopamine receptors.

Publications

Pert A. and Bunney, W.E., Jr.: Chronic lithium modulates neurotransmitter receptor sensitivity. In Lux, H.D., Addenboff, J.B., and Enrich, H.M. (Eds.): Brain Mechanisms in the Action of Lithium. Amsterdam, Excerpta Medica, 1982, in press.

Hruska, R., Pert, A., and Bunney, W.E., Jr.: Effects of lithium on muscarinic cholinergic receptors. Am. J. Pharmacol., in press.

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|---|---|--|----------------------|--------------|---------|---------------------|----------------|---------|------------|----------------|----------|---------------------|--------------------|---------|------------------|--------------|----------|----------------|-------|----------|---------------|--------------------|---------|-------------|--------------|---------|--------------|--------------|---------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <p style="text-align: center;">Z01 MH 00147-07 BP</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Behavioral and Physiological Effects of Brain Peptides and Other Psychoactive Compounds | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">P.I. Agu Pert, Ph.D.</td> <td style="width: 40%;">Psychologist</td> <td style="width: 20%;">BP NIMH</td> </tr> <tr> <td>Candace Pert, Ph.D.</td> <td>Pharmacologist</td> <td>NB NIMH</td> </tr> <tr> <td>Paul Gross</td> <td>Pharmacologist</td> <td>LCM NIMH</td> </tr> <tr> <td>Henry Holcomb, M.D.</td> <td>Clinical Associate</td> <td>BP NIMH</td> </tr> <tr> <td>Murako Kodekorno</td> <td>Staff Fellow</td> <td>LCM NIMH</td> </tr> <tr> <td>Louis Sokoloff</td> <td>Chief</td> <td>LCM NIMH</td> </tr> <tr> <td>Daniel Hommer</td> <td>Clinical Associate</td> <td>NB NIMH</td> </tr> <tr> <td>Wayne Bowen</td> <td>Staff Fellow</td> <td>NB NIMH</td> </tr> <tr> <td>Remi Quirion</td> <td>Staff Fellow</td> <td>NB NIMH</td> </tr> </table> | | | P.I. Agu Pert, Ph.D. | Psychologist | BP NIMH | Candace Pert, Ph.D. | Pharmacologist | NB NIMH | Paul Gross | Pharmacologist | LCM NIMH | Henry Holcomb, M.D. | Clinical Associate | BP NIMH | Murako Kodekorno | Staff Fellow | LCM NIMH | Louis Sokoloff | Chief | LCM NIMH | Daniel Hommer | Clinical Associate | NB NIMH | Wayne Bowen | Staff Fellow | NB NIMH | Remi Quirion | Staff Fellow | NB NIMH |
| P.I. Agu Pert, Ph.D. | Psychologist | BP NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Candace Pert, Ph.D. | Pharmacologist | NB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Paul Gross | Pharmacologist | LCM NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Henry Holcomb, M.D. | Clinical Associate | BP NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Murako Kodekorno | Staff Fellow | LCM NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Louis Sokoloff | Chief | LCM NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Daniel Hommer | Clinical Associate | NB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Wayne Bowen | Staff Fellow | NB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Remi Quirion | Staff Fellow | NB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, NIMH Neuroscience Branch, NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Biochemistry and Pharmacology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2.0 | PROFESSIONAL: 1.5 | OTHER: 0.5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Microinjections of neurotensin into the periaqueductal gray matter (PAG) produced profound <u>analgesia</u> in the rat. This effect was accompanied by <u>activation</u> of neurons in the <u>raphe magnus</u> region and was attenuated by radio frequency lesions of this area. <u>Opiates</u> were found to increase the firing of <u>zona compacta dopamine</u> cells and decrease firing of <u>zona reticulata</u> cells following systemic administration. Micropressure ejection of opiates onto <u>zona compacta</u> cells had no effect while application to <u>zona reticulata</u> cells decreased their firing rate. <u>Phencyclidine</u> was found to activate the <u>nigrostriatal dopamine</u> pathways by an action in the <u>substantia nigra</u>. <u>Type 1 opiate receptors</u> were found to be localized on <u>dopaminergic terminals</u> in the <u>striatum</u>, while <u>type 2 opiate receptors</u> were found to be present on <u>cell bodies intrinsic</u> to this region. <u>Neurotensin receptors</u> were also found to be present on <u>DA nerve terminals</u> in the <u>striatum</u>, but not in the <u>nucleus accumbens</u>. <u>Electrical stimulation</u> of the <u>substantia nigra</u> elevated <u>glucose utilization</u> in the <u>subthalamic nucleus</u>, <u>entopeduncular nucleus</u> and <u>globus pallidus</u>.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Objectives

The objectives of this project are to define the mechanisms and sites of action of opiate and non-opiate neuropeptides as well as other psychoactive substances in the central nervous system.

Analgesic Effects of Opiate and Non-opiate Neuropeptides

Opiate peptides as well as opiate alkaloids are known to induce their analgesic effects through the periaqueductal gray matter (PAG). For example, injections of opiates into the PAG, which is high in opiate receptors and opiate peptides, produce profound analgesia in the rat. Besides endorphins, the PAG also contains relatively high concentrations of other neuropeptides. One neuropeptide of specific interest as a possible candidate for modulating pain transmission in this brain region is neurotensin. In order to assess the analgesic effects of neurotensin, rats were prepared with chronic indwelling cannulae guides through which neurotensin as well as other peptides could be injected into the PAG. Analgesia was assessed with both the tail-flick and hot-plate procedures. In order to ascertain whether neurotensin analgesia was mediated through descending serotonergic or noradrenergic pathways, selective lesions were made in the raphe magnus region by injection of 5,7 DHT or by radio frequency techniques. Lesions of the descending noradrenergic pathways were made by injections of 6-OHDA into the A-1 catecholamine complex. Neurotensin analgesia induced by injections into the PAG was assessed following such lesions. Electrophysiological techniques were also employed to evaluate the effects of PAG neurotensin on activity of raphe magnus cells as well as cells in the PAG.

Modulation of Nigrostriatal Dopamine Activity by Opiates

There is considerable evidence to suggest that opiates exert an important influence on the nigrostriatal dopamine system. The aim of this project was to localize and analyze the interactive effects between opiates and nigrostriatal dopaminergic neurotransmission using electrophysiological, biochemical and behavioral techniques.

Modulation of Nigrostriatal Dopamine Activity by Phencyclidine

There is evidence to suggest that some of the pharmacological effects of phencyclidine (PCP) are mediated through the dopamine system. While PCP has been shown to influence dopaminergic activity, the loci of such actions are unknown. Of interest is the finding that the caudate nucleus as well as the substantia nigra (SN) contain relatively high concentrations of PCP receptors. The purpose of these studies was to ascertain whether the effects of PCP on dopaminergic activity are mediated through either of these structures. In the first series of studies, rats were lesioned unilaterally in the SN with 6-hydroxydopamine. Two to three weeks later the animals were tested in an automated rotometer following i.p. injections of 3, 10 and 20 mg/kg PCP. In order to localize the actions of PCP on DA transmission, rats were implanted with unilateral cannulae guides aimed for either the caudate nucleus or the substantia nigra. Following recovery, the animals were injected in these structures with 3, 10 or 25 n/moles of PCP or various analogs.

Relationship of Opiate Receptors to Nigrostriatal DA Pathways

The prototype μ receptor ligand, dihydromorphine (DHM), and the prototype delta receptor ligand, [D-Ala²,D-Leu⁵]-enkephalin (D-ENK), bind to slide-mounted sections of rat striatum with distinctly different patterns. DHM binds discretely to patches (Type 1 pattern) while D-ENK binds diffusely (Type 2 pattern). However, we have found that allosteric effectors (Na+Mn+GTP) increase binding of D-ENK to patches and decrease binding of DHM without affecting D-ENK binding at diffuse sites. We have postulated that the Type 1 receptor is conformationally malleable with varying affinities for μ , κ , and δ ligands (Bowen et al. *PNAS*, 4818, 1981; Quirion et al. *Adv. Endogenous and Exogenous Opioids*, 63, 1981) while the Type 2 receptor is conformationally static and maintains a δ -like ligand selectively (Olgiati et al. *Life Sci.*, in press). Nigral 6-hydroxydopamine (6-OHDA) and striatal kainic acid (KA) lesions were used to ascertain the location of Type 1 and 2 opiate receptors on elements of striatum. Rats received unilateral injections of 6-OHDA (9 μ g) into the substantia nigra or KA (1 μ g) into the striatum and 4 and 6 weeks, respectively, were allowed to elapse before sacrifice of animals. Sections of striatum were prepared and incubated with ³H-DHM and ³H-D-ENK under conditions which label Type 1 receptors with highest affinity (Type 1 conditions): Mn for DHM and Na+Mn+GTP for D-ENK. Type 2 receptors were labeled by ³H-D-ENK in presence of Mn + unlabeled naloxone (Type 2 condition), which minimizes binding to Type 1 receptors. (Concentrations are: NaCl, 100 mM; Mn(OAc)₂, 3 mM; naloxone, 2 nM; ³H-DHM, 1 nM; ³H-D-ENK, 2.5 nM.) Sections were then subjected to autoradiography and the lesioned side compared to the unlesioned side by grain count analysis or computed optical density analysis.

Relationship of Neurotensin Receptors to DA Pathways

Neurotensin has been shown to exert complex effects on dopamine neurotransmission. These effects appear to be mediated through dopaminergic cell body areas as well as their terminal fields. The purpose of this study was to ascertain the precise localization of neurotensin receptors in relation to mesolimbic and nigrostriatal dopamine neurons. Nigrostriatal dopaminergic projections were lesioned unilaterally by intranigral injections of 6-hydroxydopamine (9 μ g). Mesolimbic dopaminergic projections were lesioned either unilaterally (7.5 μ g 6-OHDA) or bilaterally (7.5 μ g 6-OHDA per side) by injections of this neurotoxin into the A-10 catecholamine nuclei. One month later the animals were killed and their brains removed. Serial 25 μ m sections were taken through the forebrain region encompassing the nucleus accumbens and caudate nucleus, and the midbrain region encompassing the substantia nigra and ventral tegmental area. These sections were then exposed to [³H]-neurotensin and processed for autoradiography using a tritium sensitive film. Computer optical density analysis was used to quantify the lesion effects.

Metabolic Mapping of Nigral Connections with the 2-[¹⁴C] Deoxyglucose Method

Electrical stimulation of inputs to neuronal structures has been shown to increase their rate of glucose utilization. By stimulating the substantia nigra and medial forebrain bundle, we have mapped functional neuronal circuits with the 2-[¹⁴C]deoxyglucose method. Male Sprague-Dawley rats were implanted with chronic electrodes. Following recovery, the animals were stimulated through their

electrodes with trains of biphasic pulses of 750-1000 μ A, 100 Hz, 50 msec. Rate of glucose utilization in various brain structures was assessed by standard 2-DG techniques during each stimulation.

Major Findings

Neurotensin was found to produce profound dose-dependent analgesia in the rat following direct injections into the periaqueductal gray matter (PAG). This analgesic effect was not attenuated by 5,7-DHT lesions of the raphe magnus which depleted spinal cord serotonin by 70-80%. This indicates that descending 5-HT systems are probably not activated by neurotensin in the PAG. Single unit recording studies, however, revealed that microinjections of neurotensin into the PAG produced a dramatic increase in the local firing rates of neurons in the raphe magnus region. These cells were probably not serotonergic, however. The nonserotonergic competition of the hindbrain link was exemplified by the fact that radiofrequency lesions were effective in attenuating analgesia induced by injections of neurotensin into the PAG, whereas, 5,7-DHT lesions were not. One of the descending links also appears to be noradrenergic in nature since 6-OHDA lesions of the A-1 catecholamine nuclei were also effective in reducing the analgesic effects of PAG neurotensin. Neurotensin itself applied directly to neurons in the PAG with iontophoretic techniques produced significant excitation of these neurons.

Single unit recording techniques revealed that i.v. morphine increases the firing rate of zona compacta dopamine neurons in the substantia nigra (SN-DA). This increase was completely reversed by 0.01 mg/kg of i.v. naloxone. On the other hand, zona reticulata cells (SN-ZR) were clearly inhibited by i.v. morphine. These SN-ZR neurons were more sensitive to morphine than SN-DA neurons and decreased their firing rate an average of 26% below baseline following 1.0 mg/kg of morphine. Micro-pressure ejection of either morphine or D-al²-D-leu⁵-enkephalin (DADL) had no effect on the basal activity of zona compacta neurons. The basal activity of neurons in the zona reticulata which could be activated by noxious input was inhibited by direct application of DADL. This class of neurons are inhibitory in nature and are thought to project to the zona compacta cells. Opiates in the substantia nigra appear to excite zona compacta neurons through disinhibition.

PCP was found to induce dose-dependent ipsilateral rotational behavior. This effect was attenuated by microinjections of haloperidol (10 ug) into the striatum contralateral to the lesion. In the second series of studies, direct unilateral microinjections of PCP (3, 10 and 25 nmoles) into the SN induced dose-dependent rotational behavior contralateral to the injection. This effect was attenuated by injections of haloperidol into the ipsilateral striatum. Unilateral injections of +PCMP into the SN were five times as effective in eliciting rotational behavior as -PCMP. This corresponds with the efficacy of these two enantiomers in inhibiting PCP binding to brain homogenates. Unilateral injections of PCP (50 nmoles) into the caudate nucleus were without effect on rotational behavior. The findings from these studies suggest that PCP may activate the dopaminergic nigrostriatal pathways through an action in the SN. The precise mechanism of this effect is under study.

6-OHDA caused approximately a 50% decrease in both DHM and D-ENK binding to Type 1 receptor patches. Type 2 labeling by D-ENK was not affected. By contrast,

kainic acid (KA) reduced D-ENK binding 70% under Type 2 conditions and 57% under Type 1 conditions. DHM was reduced only 36% by KA under Type 1 conditions. Thus, Type 1 receptors are sensitive to 6-OHDA lesioning while Type 2 receptors are not, indicating terminal localization. Type 2 receptors are more sensitive to KA lesioning than Type 1, indicating cell body localization. These results are consistent with the notion that Type 1 receptors are post-synaptic relative to opiate neurons which have Type 2 opiate receptors on their cell bodies. Furthermore, the observation that 6-OHDA lesioning decreases both μ and δ binding equally further supports the concept of a Type 1 receptor which can assume both μ -like and δ -like conformational states.

There appeared to be considerable binding of [3 H]-neurotensin to the substantia nigra, especially the zona compacta. Neurotensin receptors appeared to increase in number along a caudal to rostral plane in the caudate nucleus. No differences were observed along the dorsal-ventral plane however. The nucleus accumbens appeared to contain 24% more neurotensin binding than the head of the caudate nucleus at the same level. Neurotensin binding was reduced significantly in the substantia nigra following 6-OHDA lesions, indicating that some neurotensin receptors are located on dopaminergic cell bodies or dendrites. Similar findings have been reported by Palacios and Kuhar (Nature, 294:587, 1981). Neurotensin binding also decreased in the more medial A-10 regions following 6-OHDA injections. We also found a substantial decrease of neurotensin receptors in the caudate nucleus ipsilateral to the nigral 6-OHDA lesions. Neurotensin binding in the dorsal half of the caudate was reduced 65% relative to the unlesioned side. Binding in the ventral aspect, however, was reduced only 37%. Interestingly, neither unilateral nor bilateral A-10 lesions were effective in reducing neurotensin binding in the nucleus accumbens. Neurotensin receptors appear to be localized on dopaminergic terminals in the nigrostriatal but not the mesolimbic pathway. Dopaminergic cell bodies giving rise to both of these pathways, on the other hand, are high in neurotensin receptors. The pharmacological effects of neurotensin on dopaminergic neurotransmission are probably mediated through all of these sites.

In male Sprague-Dawley rats with chronically implanted electrodes, unilateral stimulation of the substantia nigra (biphasic pulses of 750-1000 A, 100 Hz, 50 s) evoked characteristic behavioral responses such as ipsilateral shoulder and head rotation and forepaw planting that were synchronous with the stimulation. In contrast to sham controls, the metabolic pattern in stimulated animals displayed marked and selective increases. Glucose utilization was elevated in the ipsilateral subthalamic nucleus by 38% (contralateral: 68 ± 7 moles/100 g/min; ipsilateral: 94 ± 11 , mean \pm SE, $n=4$) and in the entopeduncular nucleus by 85% (contralateral: 55 ± 6 ; ipsilateral: 102 ± 4 , $n=4$). An increase in glucose utilization also occurred in the ipsilateral globus pallidus. To evaluate the relative contributions of orthodromic and/or antidromic activation of afferent and efferent connections of the substantia nigra, we examined the metabolic patterns resulting from unilateral stimulation of the medial forebrain bundle. In contrast with the effects of nigral stimulation, no increases in glucose metabolism were found in the ipsilateral subthalamic, entopeduncular or pallidal nuclei. A marked activation of glucose utilization was observed, however, in the ipsilateral zona compacta of the substantia nigra and along the medial forebrain bundle up to its most rostral projections. These results indicate that the regional increases in metabolism during nigral stimulation resulted from both orthodromic and antidromic ac-

tivation of nigral connections. Lesions and pharmacological manipulations should reveal the relative importance of these two mechanisms.

Significance to Biomedical Research and the Program of the Institute

Since opiates are among the most potent psychotomimetic and euphorogenic compounds, it is of special significance that there exist endogenous compounds in brain which are apparently mimicked by opiates. There is substantial evidence that these compounds may be novel neurotransmitters or neuroregulators. If this is the case, then it is quite conceivable that such neurohumors may play an important role in affective states. Other peptides in brain appear to exert important effects on dopamine neurotransmission. Since dopaminergic dysfunctions have been related to mental disorders, it is possible that various neuropeptides may be involved in the etiology of such illnesses.

Proposed Course

1. Electrophysiological and neurochemical techniques will be used to analyze the interactive effects of phencyclidine with dopamine transmission.
2. Autoradiographic and electrophysiological techniques will be used to analyze the interactive effects of various opiate ligands with the dopamine pathways.
3. Behavioral as well as neurophysiological procedures will be used to evaluate the physiological role of neurotensin in the brain.
4. Studies will be initiated, using the 2-DG technique, to ascertain which pathways in brain are functionally active during certain behavior.
5. Autoradiographic techniques will be used to evaluate changes in brain receptor binding properties following various environmental manipulations.

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Bragin, E., Mandy, T.W., Pert, C.B., and Pert, A.: Effects of stress on brain and spinal cord levels of bombesin, substance P and endorphins. Pharmacol. Biochem. Behav., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00157-04 BP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies in Benzodiazepine Binding Sites in the Central Nervous System | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: John F. Tallman OTHER: John W. Thomas Alice Smith | Chief, Section on Biochemistry and Pharmacology Chemist Biol. Lab Technician | BP NIMH BP NIMH BP NIMH |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Biochemistry and Pharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.7 | PROFESSIONAL: 0.7 | OTHER: 2.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Studies on the mechanism of action of the <u>benzodiazepines</u> indicate that benzodiazepines alter neuronal inhibition in the central nervous system through a GABA receptor/benzodiazepine/ionophore complex. The ability to covalently label this site with a radioactive benzodiazepine has resulted in labeling of the active site. The binding of benzodiazepine agonists and antagonists (anti-diazepam) compounds has been studied and the antagonist binding site has been differentiated from the agonist binding site. | | |

Many of the pharmacological actions of the benzodiazepines can be attributed to their actions at a GABA receptor--benzodiazepine binding site--ionophore complex in brain. This site is probably the major site of action of these drugs and its molecular properties have been investigated in some detail using various benzodiazepines and newly discovered antagonists of the benzodiazepines.

Both the brain-specific and peripheral-type benzodiazepine receptors have been solubilized using various detergents. The properties of these sites are being clarified. In addition, the specific photoaffinity labeling of membrane-bound and detergent-solubilized benzodiazepine binding sites has been investigated using UV irradiated [^3H]flunitrazepam as a photochemical probe. The time course and the regional and pharmacological specificity of the photolabeling reaction has been determined for "brain-specific" benzodiazepine binding sites; "peripheral-type" binding sites treated in an identical manner were not specifically labeled. Comparison of the number of sites labeled and blocked by [^3H]flunitrazepam photolabeling of detergent-solubilized preparations indicated that about one site was blocked and unavailable for reversible binding for each site photolabeled. In contrast, when membrane-bound sites were photolabeled, about four sites were inactivated for each site photolabeled. Examination of photolabeled binding sites from various brain regions including cortex, striatum, and hippocampus using SDS-PAGE gave only a single labeled band of apparent molecular weight 48,000.

To examine the benzodiazepine binding site in greater detail, the binding of several antagonists was examined. These compounds, of diverse chemical structure, have the property of reversing many of the behavioral actions of the benzodiazepines. They include several β -carbolines (derivatives of tryptophan), an imidazodiazepine, and a quinoline derivative. These compounds bind to a site quite close to the binding site for the benzodiazepines and are capable of competitively inhibiting binding to this site. On the other hand, the benzodiazepines are capable of reversibly inhibiting the binding of the antagonists to their sites. In contrast to the binding site for the benzodiazepines, the antagonist binding site is not regulated by GABA, anions and many of the other allosteric modifiers capable of enhancing benzodiazepine binding site affinity.

As mentioned above, following photolabeling, more binding sites for benzodiazepines are inactivated than labeled. In contrast, the binding site for antagonists is not altered. Thus, the photolabeled binding site is not identical to the antagonist binding site; when the benzodiazepines were used to displace antagonist binding, they were found to be much weaker than previously and the displacement curves flattened, indicating interactions with altered binding sites.

By comparing GABA activation of binding and the ability to differentially alter the binding site by photolabeling, we can make predictions about the agonist/antagonist behavioral properties.

Significance to Biomedical Research and Institute Program:

These studies have been directed towards a more complete understanding of the pharmacology and biochemistry of the GABA receptor/benzodiazepine/ionophore complex. Based on electrophysiological, receptor binding, and molecular biology studies, it is clear that the benzodiazepines alter neuronal inhibition in the central nervous system. The mechanism for this enhanced inhibition is obviously complex, but studies on membrane environment and further biochemical characterization of the isolated binding site will lead to a more comprehensive understanding of the mode of action of benzodiazepines and, perhaps, brain inhibitory mechanisms in general. Additionally, the benzodiazepines are among the most widely prescribed drugs in the world and the presence of antagonists for this site may provide new explanations for the causes of anxiety.

Proposed Course:

Continued studies on the function of the benzodiazepine binding site in neuronal inhibition are expected. We also hope to further characterize the isolated binding site and membrane environment of this receptor. A fuller development of the antagonist binding properties is anticipated.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00159-03 BP | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Neurotransmitter Receptors in the Nervous System | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Marian S. Kafka</td> <td style="width: 33%;">Physiologist</td> <td style="width: 33%;">BP NIMH</td> </tr> <tr> <td>OTHER: Marco A. Benedito</td> <td>Visiting Fellow</td> <td>BP NIMH</td> </tr> </table> | | | PI: Marian S. Kafka | Physiologist | BP NIMH | OTHER: Marco A. Benedito | Visiting Fellow | BP NIMH |
| PI: Marian S. Kafka | Physiologist | BP NIMH | | | | | | |
| OTHER: Marco A. Benedito | Visiting Fellow | BP NIMH | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | |
| SECTION Section on Biochemistry and Pharmacology | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL MANYEARS: 1.6 | PROFESSIONAL: 1.6 | OTHER: 0.0 | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) HUMAN SUBJECTS</td> <td><input type="checkbox"/> (b) HUMAN TISSUES</td> <td><input checked="" type="checkbox"/> (c) NEITHER</td> </tr> <tr> <td colspan="3"><input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS</td> </tr> </table> | | | <input type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input checked="" type="checkbox"/> (c) NEITHER | <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input checked="" type="checkbox"/> (c) NEITHER | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Neurotransmitter receptors</u> are being studied in the rat <u>central nervous system</u> . <u>Brain neurotransmitter receptors</u> undergo <u>circadian rhythms</u> which may be altered by changed physiological function, the seasons of the year, and drugs. | | | | | | | | |

Objectives:

- (1) To develop methods to measure the presence of neurotransmitter receptors in the nervous system.
- (2) To measure whether alterations in receptor number accompany alterations in function in the central nervous system.
- (3) To measure rhythms in brain receptors and study their alterations with changes in function and with drugs.

RHYTHMS IN BRAIN NEUROTRANSMITTER RECEPTORS

Methods Employed:

The specific binding of tritiated ligands to membranes prepared from rat brains is used to measure rhythmic changes in receptor binding in rats sacrificed at intervals over a 24-hour period, over the course of the year, with and without physiological alterations and drug treatment. The α -adrenergic receptor is measured by the specific binding of [^3H]WB4101; the β -adrenergic receptor, by [^3H]dihydroalprenolol; the dopamine receptor, by [^3H]spiroperidol; the muscarinic acetylcholine receptor, by [^3H]QNB; the opiate receptor, by [^3H]naloxone; and the benzodiazepine receptor, by [^3H]diazepam.

Major Findings:

- (1) There are circadian rhythms in the number of α - and β -adrenergic, dopamine, opiate, benzodiazepine, and muscarinic acetylcholine receptors in rat brain.
- (2) The circadian rhythm in each of these is changed by the chronic administration of imipramine, clorgyline, or lithium carbonate.
- (3) A single 24-hour period of sleep deprivation changed the circadian rhythms in each receptor only slightly in rat brain.

Significance to Biomedical Research and the Program of the Institute:

These experiments document the existence of circadian rhythms in some brain neurotransmitter receptors. The rhythms are in the number of receptors, not the affinity of each receptor for its tritiated ligand. As transmission across CNS synapses is modulated by receptors, the change in the number of a given receptor, both pre- and post-synaptically, as well as the relationship between that receptor and other receptors, and the relationship of all to the light-dark cycle in the environment could have a profound effect on neuronal activity patterns in the brain. In addition, information about the alteration of receptor rhythms by chronically administered psychoactive drugs may help to elucidate the therapeutic roles of these drugs in man.

Proposed Course:

The rhythms in α - and β -adrenergic, dopamine, opiate, acetylcholine, and other receptors are being measured with additional drugs and after other functional alterations. The relationship of the rhythms to the "biological clock" is being examined. The existence of receptor rhythms in more discrete brain regions and their functional correlates are being investigated.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center;">Z01 MH 00160-01 BP</div> | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Control of β -Adrenergic Receptors in Cultured Cells | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">John F. Tallman</td> <td style="width: 40%;">Chief, Section on Biochemistry and Pharmacology</td> <td style="width: 5%;"></td> </tr> <tr> <td>OTHER:</td> <td>Peter H. Fishman</td> <td>Research Biochemist</td> <td>BP NIMH</td> </tr> <tr> <td></td> <td>Craig C. Smith</td> <td>Chemist</td> <td>DMNB NINCDS BP NIMH</td> </tr> </table> | | | PI: | John F. Tallman | Chief, Section on Biochemistry and Pharmacology | | OTHER: | Peter H. Fishman | Research Biochemist | BP NIMH | | Craig C. Smith | Chemist | DMNB NINCDS BP NIMH |
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| | Craig C. Smith | Chemist | DMNB NINCDS BP NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | |
| SECTION Section on Biochemistry and Pharmacology | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.3 | PROFESSIONAL: 0.3 | OTHER: 1.0 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>β-Adrenergic receptors</u> are present in a number of cultured cell lines and their ability to function is altered by a number of factors in the culture medium and the length of time in culture. When <u>astrocytoma cells</u> (C6 glioma) undergo many generations, the ability of the cell to respond to catecholamines is profoundly reduced. This reduction in responsiveness is due to several factors including a decreased number of β-adrenergic receptors and increased phosphodiesterase activity; this results in a 5-fold decrease in cAMP half-life in the cell. Ultimately, these findings may be important in understanding age-related phenomena. When either old or young astrocytoma cells are exposed to catecholamines, a rapid and specific desensitization to a second stimulation by catecholamines occurs. This seems to occur as a two-step process. The first step involves uncoupling of β-adrenergic receptor from adenylate cyclase; the second step involves internalization and loss of receptor molecules. </p> | | | | | | | | | | | | | | |

Responsiveness to catecholamines was studied in two different strains of rat glioma C6 cells. The C6 cells of low passage possessed a high capacity to accumulate cyclic AMP in response to (-)-isoproterenol. High passage C6 cells were unresponsive to (-)-isoproterenol except in the presence of a high concentration of phosphodiesterase inhibitor. The affinity of β -adrenergic receptors on both strains for (-)-[³H]dihydroalprenolol was similar; however, C6 low passage possessed several times the number of β -adrenergic receptors found in C6 high passage. In intact cells, the rate of breakdown of cyclic AMP was 5-times faster in C6 high passage than in C6 low passage cells. Thus, differences in β -adrenergic receptor number and phosphodiesterase activity explain, in part, the lack of responsiveness of C6 high passage cells. Our studies indicate that continuous subculturing of rat glioma C6 cells led to complex alterations in the β -adrenergic receptor-adenylate cyclase system.

Catecholamine-induced desensitization was compared in the same strains of rat glioma C6 cells. When exposed to isoproterenol, C6 low passage cells accumulate high levels of cyclic AMP (3-4 nmol/mg protein), whereas C6 high passage cells do not. After prolonged exposure to isoproterenol, both C6 low passage and C6 high passage cells exhibited a diminished response when rechallenged with the agonist. The desensitization process was both time- and dose-dependent and similar parameters were observed for C6 low passage and C6 high passage cells. C6 high passage cells exposed to isoproterenol in the presence of a phosphodiesterase inhibitor initially accumulated large amounts of cyclic AMP but the inhibitor did not alter the time course of agonist-induced desensitization. In addition, the inhibitor, which by itself elevated cyclic AMP levels more than did isoproterenol by itself in C6 high passage cells, did not induce a refractory state.

Agonist-induced loss in β -adrenergic receptors was much slower than the rate of desensitization. Membranes from desensitized cells, however, exhibited an apparent lower affinity for agonist as measured by agonist displacement of labeled antagonist binding. A substantial loss of isoproterenol-stimulated adenylate cyclase activity in membranes prepared from agonist-treated C6 low passage and C6 high passage cells was observed without a significant loss in sodium fluoride-stimulated activity.

Isoproterenol-treated C6 high passage cells remained completely responsive to cholera toxin. Desensitized C6 low passage cells exhibited a reduced response to the toxin, which was partially overcome by the phosphodiesterase inhibitor. Activation of cyclase, however, was not reduced in either C6 low passage or C6 high passage cells treated with agonist and then toxin. Furthermore, cyclase was activated to the same extent when membranes from control or desensitized cells were incubated with the A₁ subunit of cholera toxin and nicotinamide adenine dinucleotide. We conclude that the large accumulation of cyclic AMP in C6 low passage cells exposed to β -agonists does not mediate the subsequent desensitization process. Instead, the initial stage of catecholamine-induced desensitization in both C6 low passage and C6 high passage cells appear to represent a specific uncoupling of the β -adrenergic receptor from a functional regulatory component of adenylate cyclase.

We have returned to examination of similar phenomena in intact systems. Clinically, clonidine has been quite effective in the treatment of withdrawal symptoms following opiate addiction. Chronic morphine, administered to rats, results in decreased norepinephrine turnover. Concomitant with this decrease, a relative supersensitivity of postsynaptic β -adrenergic and α_2 -adrenergic receptors develops. This effect takes only a few days to develop and persists as long as morphine is present. Clonidine, when administered to animals undergoing withdrawal or to humans, may have rapid effects due to its action at relatively supersensitive receptors.

Similarly monoamine oxidase (MAO) inhibitors work in the opposite fashion to decrease the metabolism of norepinephrine, resulting in increased norepinephrine at the postsynaptic synapse. Following MAO inhibition, the apparent number of adrenergic receptors decreases, resulting in subsensitivity.

These two paradigms correspond to the long-term down regulation described above. Experiments to look at immediate desensitization are more difficult and have not yet been undertaken.

Significance to Biomedical Research and to the Program of the Institute:

The advantages of a model system to study receptor dynamics and function are tremendous compared to studies with animals. As cells in culture which are readily manipulated provide a stable source of cells which respond in a consistent fashion, the effects of drugs at known concentrations at the cell surface may be studied. The examination of drug effects in the model system can lead to predictions of *in vivo* effects of these drugs in patients. It is also possible to study subsensitivity in this model system which may be related to clinical subsensitive states. Receptor molecules are being purified to examine their molecular properties and prepare antibodies.

Proposed Course:

Research is currently underway into the following questions:

1. What molecular changes occur following desensitization; do the receptors turn over?
2. Can we purify the receptors and prepare antibodies to them?

Publications:

Fishman, P.H., Mallorga, P., and Tallman, J.F.: Catecholamine-induced desensitization of adenylate cyclase in rat glioma C6 cells: evidence for a specific uncoupling of β -adrenergic receptors from a functional regulatory component of adenylate cyclase. Mol. Pharmacol. 20: 310-318, 1981.

Hamburg, M. and Tallman, J.F.: Chronic morphine administration increases the apparent number of α_2 -adrenergic receptors in rat brain. Nature 291: 493-495, 1981.

Mallorga, P., Tallman, J.F., and Fishman, P.H.: Differences in the β -adrenergic responsiveness between high and low passage rat glioma C6 cells. Biochem. Biophys. Acta. 678: 221-229, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00166-03 BP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Peptide Secretory Products of Oat Cell Carcinoma and Other Unicellular "Creatures" | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Candace B. Pert OTHERS: Desmond Carney John D. Minna Jesse Roth | Pharmacologist Clinical Associate Chief Chief | BP NIMH MO NCI MO NCI D NIADDKD |
| COOPERATING UNITS (if any) Medical Oncology Branch, NCI; Diabetes Branch, NIADDKD | | |
| LAB/BRANCH Biological Psychiatry Branch | | |
| SECTION Section on Biochemistry and Pharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 1.0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>A number of cell types sharing a common embryological origin (APUD cells) contain neuropeptides. Also, unicellular organisms secrete neuropeptides. The possibility that these neuropeptides are used for chemical communication may suggest useful strategies for cancer chemotherapy.</p> | | |

Objectives:

To explore whether single cell organisms or tissues manufacture neuropeptides. To develop an assay to detect the early stages of oat cell lung cancer by a very sensitive radioimmuno- or radioreceptor assay for bombesin.

Methods Employed:

Radioreceptor assay, HPLC and radioimmunoassay.

Major Findings:

1. Oat cell carcinoma of the lung is characterized by aberrant fetal-like cells which secrete the brain neuropeptide bombesin.
2. In patients in advanced stages of this disease, extremely elevated bombesin levels can be demonstrated in their plasma.
3. Unicellular organisms, including *e. coli* and *tetrahymena* manufacture enkephalin beta-endorphin.

Significance to Biomedical Research and to the Program of the Institute:

While the study of lung cancer at first site may not appear to be directly applicable to the mission of the National Institute of Mental Health, an interesting side effect of the terminal stages of oat cell disease are severe psychotomimetic symptoms which may be due to the activation of brain peptide receptors with the neurocirculatory products of the lung cancer cell. It seems fitting to donate the expertise of our group in peptide work to what is actually a cancer area of research.

Proposed Course:

We plan to develop a very sensitive radioreceptor assay for detecting oat cell disease in its earliest stages before metastasis has occurred while it is still possible to destroy all cells and cure the disease by chemotherapy. We are interested in exploring the possible functions of "neuropeptides" as they are used in putative communications within and among unicellular organisms for the information it might tell us about their evolution into high functions.

Publications:

Moody, T.W., Pert, C.B., Gazdar, A.F., Carney, D.N., and Minna, J.D.: High levels of intracellular bombesin characterize human small-cell lung carcinoma. Science 214: 1246-1348, 1981.

Pert, C.B. and Schumacher, U.K.: Elevated plasma bombesin in patients with extensive small cell carcinoma of the lung. Letter to the Editor, Lancet, February 27, 1982.

LeRoith, D., Liotta, A.S., Roth, J., Shiloach, J., Lewis, M.E., Pert, C.B. and Krieger, D.T.: ACTA and beta-endorphin-like materials are nature to unicellular organisms. Proc. Natl. Acad. Sci. USA, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00167-03 BP | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Neurochemical Coding of Brain Pathways Revealed by Autoradiography | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Candace B. Pert</td> <td style="width: 33%;">Pharmacologist</td> <td style="width: 33%;">BP NIMH</td> </tr> <tr> <td>OTHERS: Miles A. Herkenham</td> <td>Psychologist</td> <td>LNP NIMH</td> </tr> <tr> <td>Wayne D. Bowen</td> <td>Staff Fellow</td> <td>BP NIMH</td> </tr> <tr> <td>Remi Quirion</td> <td>Guest Worker</td> <td>BP NIMH</td> </tr> </table> | | | PI: Candace B. Pert | Pharmacologist | BP NIMH | OTHERS: Miles A. Herkenham | Psychologist | LNP NIMH | Wayne D. Bowen | Staff Fellow | BP NIMH | Remi Quirion | Guest Worker | BP NIMH |
| PI: Candace B. Pert | Pharmacologist | BP NIMH | | | | | | | | | | | | |
| OTHERS: Miles A. Herkenham | Psychologist | LNP NIMH | | | | | | | | | | | | |
| Wayne D. Bowen | Staff Fellow | BP NIMH | | | | | | | | | | | | |
| Remi Quirion | Guest Worker | BP NIMH | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Laboratory of Neurophysiology, NIMH | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | |
| SECTION Section on Biochemistry and Pharmacology | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 3.5 | PROFESSIONAL: 3.2 | OTHER: 1.3 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) We continue to study the distribution of brain <u>neurotransmitter or drug receptors</u> by our newly developed autoradiographic method. Quantitation of receptor distribution with <u>tritium-sensitive film</u> is now also possible. Specifically, chemically coded neuronal pathways can be surmised from the concordance of receptor distributions and autoradiographic track tracing techniques. Furthermore, adjacent sections can be incubated under different conditions with different ligands to reveal information about <u>receptor subtypes</u> . | | | | | | | | | | | | | | |

Objective:

To map the neuroanatomical distribution of various chemically coded pathways in brain.

Methods Employed:

1. Newly developed in vitro autoradiography--unfixed frozen brain tissue is melted onto slides, incubated in appropriate radioactive ligands to label receptors, washed thoroughly, dried rapidly, fixed with paraformaldehyde vapors and dipped in radio-sensitive liquid emulsion for traditional autoradiographic workup.
2. Receptor-labelled slides are placed tightly against tritium-sensitive film for two weeks and then grain density is transformed by computer.
3. Old-fashioned "grind and bind," whereby tissue dissection is carried out followed by homogenization and study of specific binding to homogenates after rapid filtration.

Major Findings:

1. Neurotensin receptors appear to be diminished in striatum of human brain from schizophrenics. Consistent with this finding, and interestingly, they appear to be encrusted on both the cell bodies and terminals of dopaminergic projections of rat brain.
2. Receptor localization can be combined with the 2-deoxy-glucose (2-DG) technique in the same animal by rapidly washing out 2DG and retaining receptors intact.
3. A major method paper, which discusses the two new methods for visualizing brain receptors autoradiographically, has been accepted in The Journal of Neuroscience.
4. Striatal opiate receptors show a profound evolutionary trend--the conformationally complex type 1 opiate receptor is a recent evolutionary development, while the rigid, simple type 2 opiate receptor characterizes invertebrates and lower vertebrates.
5. The phencyclidine or angel dust receptor has a cortical distribution which is suitable for receptor mediating psychotomemetic effects. The angel dust receptor has been demonstrated rigorously and stereospecific analogs further strenghtened the notion the tritiated PCP binding sites are, in fact, receptors for angel dust's psychotomimetic action.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts by this defined neuroanatomical procedure will enable us to perform lesioning and drug mimicry experiments to determine the functional significance of each newly discovered pathway. The method can be used on human brain and ultimately should give information about the contribution of various neurochemically coded tracts to pathology.

Proposed Course:

We plan to follow-up suggestions that the more complex opiate receptor is located in the most evolutionary new advanced areas of the brain, as well as being concentrated in the phylogenetically newest animals (e.g., human). We also plan to study the distribution of other receptors to reconstruct the neuronal circuitry in a similar manner.

Publications:

Quirion, R., Rice, K.C., Skolnick, P., Paul, S., and Pert, C.B. Stereospecific displacement of [³]phencyclidine (PCP) receptor binding by an enantiomeric pair of PCP analogs. Eur. J. Pharmacol. 74: 107-108, 1981.

Herkenham, M. and Pert, C.B. Light microscopic localization of brain opiate receptors: a general autoradiographic method which preserves tissue quality. J. Neurosci., in press.

Moon-Edley, S., Hall, L., Herkenham, M., and Pert, C.B.: Evolution of striatal opiate receptors. Brain Res., in press.

Quirion, R., Hammer, R.P., Jr., Herkenham, M., and Pert, C.B.: The phencyclidine (angel dust)/ σ "opiate" receptor. In: Proceedings of the 43rd Annual Scientific Meeting of the Committee on Problems of Drug Dependence, in press.

Quirion, R., Pert, C.B., Pert, A., Herkenham, M., Larssen, A., Chase, T., Wyatt, R., and Kleinman, J.: Visualization of neurotensin receptors in human and rat brain. Symposium on Neurotensin, Natl. Acad. Sci. NY, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00169-02 BP | | | | | | |
| PERIOD COVERED <u>October 1, 1981 through September 30, 1982</u> | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <u>Allosteric Receptor Modulation and Altered Sensitivity States</u> | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Candace B. Pert</td> <td style="width: 40%;">Pharmacologist</td> <td style="width: 20%;">BP NIMH</td> </tr> <tr> <td>OTHER: Uwe Kurt Schumacher</td> <td>Chemist</td> <td>BP NIMH</td> </tr> </table> | | | PI: Candace B. Pert | Pharmacologist | BP NIMH | OTHER: Uwe Kurt Schumacher | Chemist | BP NIMH |
| PI: Candace B. Pert | Pharmacologist | BP NIMH | | | | | | |
| OTHER: Uwe Kurt Schumacher | Chemist | BP NIMH | | | | | | |
| COOPERATING UNITS (if any) <u>Diabetes Research Laboratory, Veterans' Administration Hospital</u> | | | | | | | | |
| LAB/BRANCH <u>Biological Psychiatry Branch</u> | | | | | | | | |
| SECTION <u>Section on Biochemistry and Pharmacology</u> | | | | | | | | |
| INSTITUTE AND LOCATION <u>NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</u> | | | | | | | | |
| TOTAL MANYEARS: <u>1.7</u> | PROFESSIONAL: <u>1.4</u> | OTHER: <u>1.3</u> | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Neurotransmitter receptors appear to be capable of profound conformational mobility, as they couple to effectors in the cell membrane. Often, the writhings of the flexible receptor protein molecule result in alterations in ionic flux. The conformational state of receptors differ in altered states of super- and subsensitivity.</u> | | | | | | | | |

Objectives:

To demonstrate how opiate receptors can subtly shift their ligand selectivity pattern as they change their conformations when coupling with other membrane components such as adenylate cyclase. To demonstrate "down-regulation" or subsensitivity of other receptors such as the phencyclidine receptor, previously considered the sigma opiate receptor.

Methods Employed:

Radioreceptor assay and biochemical modification of proteins.

Major Findings:

1. There is an interconversion between mu and delta opiate receptors, which is mediated by the breakage and reformation of sulfhydryl bonds.
2. So-called kappa opiate receptors are really merely a fleeting, transient conformation of the conformationally complex type 1 opiate receptor, since it is found in precisely the same regions of rat brain as mu agonist and antagonist.
3. Opiate receptors appear to share the ability to regulate the same adenylate cyclase with dopamine receptors. "Plugging in and out" in a reciprocal fashion may be one mechanism of receptor super- and subsensitivity.
4. Chronic phencyclidine treatment causes a down-regulation of PCP and dopamine receptors in rat brain.

Significance to Biomedical Research and to the Program of the Institute:

The notion that alterations in mood are a function of oscillations in neurotransmitter receptor sensitivity is perhaps the most exciting new lead in attempting to understand the causes of mental illness. Studies aimed at elucidating the molecular mechanism of receptor super- and subsensitivity are thus extremely relevant to understanding psychiatric disease.

Proposed Course:

We plan future experiments to explore the relationship between the conformational state of the opiate receptor as deduced by its ligand selectivity pattern and the state of its receptor sensitivity.

Publications:

Quirion, R., Bowen, W.D., and Pert, C.B.: Mu, delta and kappa opiate receptors: interconvertible forms of the same receptors. In Advances in Endogenous and Exogenous Opioids. Proceedings of the International Narcotic Research Conference. Tokyo, Japan, Kodansha LTD., 1981, pp. 63-65.

Bowen, W. and Pert, C.B.: Conformational mobility of opiate receptors: sulfhydryl modification alters ion-induced μ/δ ligand selectivity shifts in rat striatal sections. Cell. Mol. Biol., in press.

Gentleman, S., Parenti, M., Neff, N.H., and Pert, C.B.: Inhibition of dopamine-activated adenylate cyclase and D1 receptor binding by opiate receptors in rat. Mol. Pharmacol., in press.

Quirion, R., Bayorh, M.A., Zerbe, R.L., and Pert, C.B.: Chronic phencyclidine treatment decreases phencyclidine and dopamine receptors in rat brain. Pharmacol. Biochem. Behav., in press.

Quirion, R., Herkenham, M., and Pert, C.B.: Visualization of ^3H -ethylketocyclazocine-labelled opiate receptors with a " κ " ligand selectivity pattern. Proc. Natl. Acad. Sci. USA, in press.

Quirion, R. and Pert, C.B.: Dynorphins: similar relative potencies on μ , δ and κ opiate receptors. Eur. J. Pharmacol., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00179-01 BP | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Morphological and Functional Aspects of Peptides in Mammalian Brain | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Lana Skirboll</td> <td style="width: 30%;">Staff Fellow</td> <td style="width: 15%;">Section on Biochemistry and Pharmacology</td> <td style="width: 5%;">BP, NIMH</td> </tr> <tr> <td></td> <td>Daniel Hommer</td> <td>Staff Psychiatrist</td> <td>Unit on Neuropsychopharmacology</td> <td>BP, NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Steven M. Paul</td> <td>Chief</td> <td>Unit on Preclinical Pharmacology</td> <td>CP, NIMH</td> </tr> <tr> <td></td> <td>Agu Pert</td> <td>Research Pharmacologist</td> <td>Section on Biochemistry and Pharmacology</td> <td>BP, NIMH</td> </tr> </table> | | | PI: | Lana Skirboll | Staff Fellow | Section on Biochemistry and Pharmacology | BP, NIMH | | Daniel Hommer | Staff Psychiatrist | Unit on Neuropsychopharmacology | BP, NIMH | OTHER: | Steven M. Paul | Chief | Unit on Preclinical Pharmacology | CP, NIMH | | Agu Pert | Research Pharmacologist | Section on Biochemistry and Pharmacology | BP, NIMH |
| PI: | Lana Skirboll | Staff Fellow | Section on Biochemistry and Pharmacology | BP, NIMH | | | | | | | | | | | | | | | | | | |
| | Daniel Hommer | Staff Psychiatrist | Unit on Neuropsychopharmacology | BP, NIMH | | | | | | | | | | | | | | | | | | |
| OTHER: | Steven M. Paul | Chief | Unit on Preclinical Pharmacology | CP, NIMH | | | | | | | | | | | | | | | | | | |
| | Agu Pert | Research Pharmacologist | Section on Biochemistry and Pharmacology | BP, NIMH | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Unit on Preclinical Pharmacology, Clinical Psychobiology Branch, NIMH | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Biological Psychiatry Branch | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Biochemistry and Pharmacology | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Using immunocytochemistry we have given evidence that there are several systems in the brain in which there are coexisting transmitters, i.e., more than one transmitter in a single cell. In the mesencephalon, cholecystokinin (CCK) and dopamine (DA) exist in cells of the ventral tegmentum and lateral substantia nigra zona compacta, and in the periaqueductal grey, CCK and substance P coexist in neurons. Electrophysiological studies have shown that CCK is a potent excitatory agent in substantia nigra and this excitation is limited to areas in which CCK and DA coexist. In contrast, enkephalins excite DA cells by acting indirectly on cells of the zona reticulata which normally inhibit DA cell activity. | | | | | | | | | | | | | | | | | | | | | | |

The recent surge of interest in peptide neurotransmitters has been stimulated by evidence that a number of hormones previously thought to be restricted to the gastrointestinal system are also present in nervous tissue. Isolation of these peptides and the subsequent production of antisera to these compounds has opened up possibilities to map neurons containing small biologically active peptides in brain. We have been studying the presence of such putative transmitter systems by employing the technique of immunocytochemistry. Since nerve cells are in most instances too small to allow biochemical measurements of a peptide in a single cell, the immunochemical technique permits visualization of the presence of peptides in neuroanatomically defined areas of mammalian brain. Thus, using antisera to a variety of peptides, classical transmitters, and synthetic enzymes, we have determined which cells contain a particular transmitter(s).

More specifically, animals are perfused and the sections of brain tissue are cut and stained with antisera conjugated to a fluorescent molecule to permit visualization of transmitters in single cells under the fluorescence microscope. This technique has led to the establishment of the presence of more than one neurotransmitter in a single neuron. Using the immunocytochemical technique consecutive antisera are either eluted and restained or serial sections are taken through a single cell to establish presence of more than one transmitter substance. Such experiments have revealed that a CCK-like peptide coexists in cells of the mesencephalon of rat brain which also contain DA. Similarly, we have shown that cells in the mesencephalic periaqueductal grey contain two peptides, CCK and substance P.

Once it had been established that a particular coexisting system was present at a specific neuroanatomical locus in brain, we explored the projection areas of these dual transmitter systems. For example, CCK-DA cells have been shown to be limited to the mesencephalon in areas, that are thought to project primarily to the limbic system. In an effort to address the question of whether these projections are indeed limited to limbic areas we developed a method by which retrogradely transported fluorescent dyes can be injected into nerve terminal areas, taken up into axons, transported back to cells of origin and visualized in the fluorescence microscope. Once the dye is viewed, these same sections can subsequently be stained for immunocytochemical identification of antisera, thus permitting transmitter-specific mapping of neuron projections. Using this technique we have established that CCK-DA cells project solely to limbic areas such as the central nucleus of the amygdala, stria terminalis, and accumbens. In similar studies we have shown that the CCK-SP system projects to the dorsal horn of the spinal cord while an ACTH-beta endorphin system appears to project to the ventral horn of the cord.

Our understanding of the functional significance of these coexistence systems is at present incomplete. Since most often coexisting and non-coexisting systems are intermingled in single neuroanatomical structures, it is difficult to address what function such systems might play in brain function. In an effort to elucidate what physiological role such systems might play, we have employed electrophysiological techniques to look at the effects of peptides which do coexist with classical transmitter systems such as the CCK-DA system, as well as noncoexisting systems which impinge upon classical transmitter systems such as the effect of opioids on DA cell activity. Using extracellular single unit recording from cells

in the substantia nigra zona compacta, we have found that CCK increases the firing rate and bursting activity of a particular subpopulation of cells in the mesencephalon of rat brain. This responsiveness to CCK was found exclusively in areas which were subsequently shown, using immunocytochemistry, to contain both CCK and DA. This peptide had no effect on the firing of neurons in the zona reticulata, an area devoid of CCK, which is thought to control firing of DA neurons as a part of a feedback system from the basal ganglia. In contrast, the administration of opioids increased the activity of DA cells indirectly by altering activity of cells in the reticulata.

Projected Course:

We plan to continue looking at the physiological significance of coexistence using electrophysiological techniques. We will primarily be exploring two systems: there is evidence for an avian pancreatic polypeptide (APP)-like peptide in the locus coeruleus of the rat. Given the important role of this nucleus in control of noradrenergic and adrenergic function we will explore the effects of this peptide. Recently we have found that this substance coexists with adrenaline in cells of the C1 area of brain and that these systems may well project to the locus. Thus, we plan to explore these projections using retrograde tracing with immunocytochemistry and extend these morphological studies to examine the electrophysiology of these two potentially interacting transmitters. We also plan to actively continue our work with the CCK-DA system by looking at the interaction between these two transmitters at one of the limbic system nerve terminal areas, specifically the nucleus accumbens.

Significance to Biomedical Research and to the Program of the Institute:

Identification of more and more peptide putative transmitters in brain has permitted elucidation of a more refined network in mammalian nervous tissue. The use of immunocytochemical techniques is limited only by the ability to raise antisera to a specific antigen. Thus, such techniques will continue to reveal more about functional transmitter pathways in brain. The immunocytochemical data can, however, no longer stand alone. The functional significance of this multitude of transmitter candidates can only be explored using techniques such as electrophysiology, i.e., looking at the effects of specific peptides and transmitter combinations on the activity of single well-defined cells in brain. Such combined information, using information from immunocytochemistry on transmitter identification and electrophysiology on function, may lead to a better understanding of chemical transmission in the nervous system. It is hoped that the function of coexisting systems may ultimately permit the development of drugs which have a more selective action on specific subpopulations of cells which may be altered in neurological and mental disease. An example of this may be seen with the CCK-DA system and the potential role of a peptide which may interact solely with limbic function and thus play a role in the etiology and symptomatology of schizophrenia.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00326-09 CN | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Clinical Neuropharmacology and Psychobiology of Mania and Depression</p> | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> PI: Dennis L. Murphy OTHER: Robert M. Cohen Thomas Insel Laurence Guttmacher Jean Hamilton Larry J. Siever Benjamin Roy Carol F. Hoover Edward F. Donnelly Stanley L. Slater </td> <td style="width: 30%; vertical-align: top; border: none;"> Chief Staff Physician Staff Physician Staff Physician Staff Physician Staff Physician Staff Physician Social Worker Psychologist Staff Physician </td> <td style="width: 20%; vertical-align: top; border: none;"> CN NIMH CN NIMH CN NIMH CN NIMH CN NIMH CN NIMH CN NIMH SMR NIMH DIR NICHD </td> </tr> </table> | | | PI: Dennis L. Murphy OTHER: Robert M. Cohen Thomas Insel Laurence Guttmacher Jean Hamilton Larry J. Siever Benjamin Roy Carol F. Hoover Edward F. Donnelly Stanley L. Slater | Chief Staff Physician Staff Physician Staff Physician Staff Physician Staff Physician Staff Physician Social Worker Psychologist Staff Physician | CN NIMH CN NIMH CN NIMH CN NIMH CN NIMH CN NIMH CN NIMH SMR NIMH DIR NICHD |
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| COOPERATING UNITS (if any) <p style="text-align: center;">Iain C. Campbell, Maudsley Hospital, London, England</p> | | | | | |
| LAB/BRANCH <p style="text-align: center;">Clinical Neuropharmacology Branch</p> | | | | | |
| SECTION | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) <p>Investigation of the behavioral and physiological consequences of treatment with <u>monoamine oxidase</u> inhibiting antidepressants have revealed a clear temporal <u>dissociation between</u> the rapid development of enzyme inhibition and the delayed onset of the remission of depressive symptoms, the precipitation of hypomanic or <u>manic episodes</u> and reductions in <u>rapid-eye movement sleep</u> and in <u>blood pressure</u>. Significant associations between orthostatic blood pressure changes and anti-depressant effects, together with evidence that central <u>α-adrenergic receptor</u> changes (as assessed by cardiovascular responses to <u>clonidine</u>) are also delayed, suggest that adaptive alterations in brain <u>noradrenergic function</u> may be an important mechanism in the therapeutic actions of these drugs. In other studies, baseline growth hormone elevations in response to clonidine administration were found to be significantly blunted in depressed patients compared to sex- and age-matched controls, suggesting the possibility of a primary abnormality in the noradrenergic system in these patients.</p> | | | | | |

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with schizoaffective and characterologic disorders, are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients' self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed on this unit.

2. Biological assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of enzymes, levels of biogenic amines and their metabolites, other chemical variables, and drug levels. Electro-physiologic measurement of sleep and of cortical evoked potentials are accomplished in collaborative studies with investigators in the Biological Psychiatry Branch. Pharmacologic challenge tests of central neurotransmitter systems are also used to evaluate the functional state of these systems in affective disorder patients compared to controls and in patient groups studied before and during antidepressant drug treatment.

Major Findings:

Delayed behavioral consequences of antidepressant drug treatment: Further studies of the effects of the selective monoamine oxidase (MAO) inhibitors, clorgyline and pargyline, have explored some delayed physiological consequences of their administration which, to a varying extent, correlate with their antidepressant effects. These drugs, like the tricyclic antidepressants, manifest a several-week delay in onset of their therapeutic actions despite full-dose administration and despite evidence from platelet MAO activity measurements and plasma and urinary amine metabolite changes that nearly complete enzyme inhibition is present after only a few days of drug treatment.

A similar delay in onset of an occasional side effect of MAO-inhibitor administration, the precipitation of hypomanic or manic episodes, was also regularly found to be the case according to a retrospective review conducted by David Pickar. All of the patients who developed this side effect in the clorgyline-pargyline trials, as well as those in a previous study on the same research unit with another MAO inhibitor, phenelzine, did so after a minimum of

22 days of drug administration. The only exception to this time lag occurred in a few patients who had received an MAO-inhibitor less than three weeks before beginning of the second trial with a different MAO inhibitor.

Sleep changes during longer-term MAO-inhibitor administration: The most marked effect of most MAO inhibitors on EEG-monitored sleep patterns is a marked, near total suppression of rapid eye movement (REM) sleep. This change has been noted previously with non-selective MAO inhibitors such as phenelzine, but not with the MAO-B selective inhibitor, deprenyl, which produced only a 15% reduction in REM sleep in one report in the literature. In a recently completed analysis of sleep recordings made during selective MAO inhibitor administration, both clorgyline and high doses of pargyline produced essentially complete (97-100%) REM sleep suppression. This effect typically took 7 to 10 days after the initiation of drug administration to become complete. Similarly, there was a 7- to 10-day lag period after stopping the MAO inhibitor before REM sleep returned to pretreatment levels. It should be noted that although the time course for this change in REM sleep is clearly delayed beyond the 4-24 hour period needed for MAO inhibition to become essentially (>90%) complete, the REM sleep alteration occurs earlier than the mood alterations, raising the question of a sequence of changes involving possibly different mechanisms being required for these different drug effects.

Cardiovascular changes during longer-term MAO-inhibitor administration: A time lag in the onset of other clinical phenomena besides the mood and sleep changes was also observed with these MAO inhibitors. Clorgyline treatment in these patients led to reductions in systolic, diastolic and mean arterial blood pressure. Lesser blood pressure changes occurred during pargyline and deprenyl administration. Greater changes were observed in blood pressures measured when standing than sitting. This orthostatic hypotension, which was clinically symptomatic in approximately one-fourth of the patients, developed gradually during the 4-week period of clorgyline administration, with the greatest difference between sitting and standing pressures becoming evident in the fourth week of drug treatment and in the subsequent week following clorgyline discontinuation. The time course of the onset of the blood pressure changes thus tended to parallel the time course of onset of clinical antidepressant effects. In addition, following discontinuation of clorgyline, depressive symptoms began to reappear and blood pressure returned towards pretreatment levels after a 7-10 day lag time following cessation of treatment.

Some further evidence for an association between the blood pressure reductions and clinical antidepressant effects was found in the significant positive correlation ($r = + 0.58$, $p < .05$) between the reduction in Hamilton depression scale ratings and the reduction in standing blood pressure in the final week of clorgyline administration. Correlation coefficients of similar magnitude between blood pressure changes and therapeutic effects have also been found for several of the self-rated scales used in this study, including the Beck Depression Scale and the POMS depression factors.

Changes in the pressor response to clonidine during longer-term antidepressant drug administration: A more direct examination of the question of possible processes involved in the different time-dependent effects of clorgyline has recently been conducted by Larry Siever using a pharmacologic challenge approach to assess the status of the noradrenergic neurotransmitter system in man. This particular study utilized clonidine, a centrally-acting adrenergic agent which in low doses produces a hypotensive response. This blood pressure lowering effect appears due to clonidine's action as an α_2 -adrenergic agonist since this hypotensive response can be induced in animals by local application of clonidine to the locus ceruleus, where α_2 -adrenergic autoreceptors mediate an inhibitory feedback on noradrenergic activity.

Depressed patients examined prior to clorgyline administration manifested a reduction in mean arterial pressure following a single clonidine dose. The maximum reduction was attained approximately one hour after administration. When clonidine administration was repeated after three weeks' treatment with clorgyline, nearly complete blockade of the clonidine hypotensive response occurred. However, after clorgyline administration for only three days, a hypotensive response equal to that found in the control, pre-clorgyline period was observed. Overall, a consistent, highly statistically significant attenuation of the clonidine response has been found during longer-term but not short-term clorgyline administration to nine patients, with an intermediate level of blockade observed after ten days of treatment. This data provides evidence that the antagonism of the clonidine response may well represent a subsensitization of brain α_2 -adrenergic receptors resulting from chronic but not acute monoamine oxidase inhibition. This would have the effect of lessening the feedback inhibitory impact of the increased norepinephrine availability induced by clorgyline. The cellular basis of these changes as examined in animal studies is discussed in another project report by Robert M. Cohen (Z01 MH 00332-04). Some of these slowly-developing physiological changes may be of pertinence to the observations reported this year by William Potter that clorgyline in low doses had a stabilizing effect in patients with previously drug-resistant forms of bipolar affective disorder characterized by rapid cycles between depression and mania.

Direct assessment of the status of noradrenergic receptors in depressed patients: One study, performed by Larry Siever in collaboration with Thomas Uhde and Robert Post, has been using clonidine, a selective α_2 -adrenergic agonist, as a pharmacologic challenge of the noradrenergic system in affectively disordered and obsessive-compulsive patients prior to and during antidepressant treatment. Clonidine stimulates growth hormone release by acting on post-synaptic α_2 -adrenergic hypothalamic receptors; the plasma growth hormone increase produced by clonidine was found to be blunted in most depressed patients, suggesting decreased responsiveness of these central α_2 -adrenergic receptors.

In addition, the magnitude of the growth hormone response has been found to correspond inversely to pretreatment plasma MHPG concentrations in these patients, suggesting that increased noradrenergic release may be associated with decreased α_2 -adrenergic responsiveness, a finding predicted from basic principles of receptor adaptation. We also have preliminary evidence that

clonidine may induce a decrement in plasma cortisol concentrations, and that this decrement may be greater in depressed patients than controls. We are pursuing this lead to further explore the relation of plasma cortisol to the noradrenergic system.

We are also conducting a pilot study in collaboration with Craig Risch, of the relationship of the neuroendocrine response to clonidine and the behavioral and neuroendocrine responses to arecholine, a cholinergic agonist. Preliminary data obtained last year suggested the possibility that patients and controls with a decreased response to clonidine may be supersensitive to arecholine, consistent with the cholinergic-adrenergic balance hypothesis of the affective disorders. Platelet α_2 -adrenergic receptor status in depressed patients is also being evaluated by Larry Siever in collaboration with Marian Kafka. Increases in α_2 -adrenergic receptor binding have been observed in unipolar depressed patients. In an attempt to explain these paradigms in relation to the serotonergic system, we are completing a study of the prolactin response to fenfluramine in depressed patients and controls started by Stanley Slater.

Affective symptoms associated with the menstrual cycle: Jean Hamilton initiated a series of investigations of menstrual-cycle related affective disorder during the last year. Approximately 11 subjects (5 with affective disorder) with self-identified premenstrual affective symptoms have entered the program through the outpatient clinic or the inpatient unit, while another group of 8 symptomatic subjects were identified from an initial sample of 97 women volunteers who were screened for the presence of menstrual-related affective symptoms apart from other evidence to psychopathology. Another 16 non-symptomatic volunteers and screening subjects form a third comparison group.

Subjects in these index and control groups have been evaluated on a number of mood, behavioral, biological and clinical measures in the premenstrual versus intermenstrual phases of the cycle. Although only preliminary data are available, it appears that the self-identified population may differ from the screening group when compared on daily mood and personality characteristics. In other investigations with Larry Siever, neuroendocrine responsivity to clonidine versus placebo challenges in pre- and post-menstrual phases of the cycle are being compared across subject groups as possible aids in identifying subgroups. In view of the Reid and Yen hypothesis linking β -endorphins to premenstrual symptoms and the finding that clonidine suppresses opiate withdrawal symptoms, these studies may have implications for developing approaches to treatment. Since we are using the night-time melatonin response to clonidine as an endpoint measure, another project has included the evaluation of the natural pattern of urinary 6-hydroxy melatonin excretion across the menstrual cycle in six normal subjects, in collaboration with Sanford Markey.

Additional studies: Other projects dealing with the assessment and treatment of neuropharmacologic patients with affective disorders included the completion of a major review of rating scale methods for the evaluation of depressive and manic symptoms and the completion of a series of reviews of the pharmacological challenge approach to evaluating the functional status of the different central neurotransmitter and neuromodulator systems in man. Several reviews of various aspects of the clinical uses of monoamine oxidase inhibiting

antidepressants were also completed, along with a review of the question of possible relationships between antidepressant drug responsiveness and diagnostic subgroups among affective disorder patient populations.

Significance to Biomedical Research and the Program of the Institute:

Our major body of studies using monoamine oxidase (MAO) inhibiting antidepressants (and in a few cases, tricyclic antidepressants) have provided one of the most detailed bodies of evidence in the literature demonstrating that the well-known delay in onset of clinical antidepressant effects is separable from certain biochemical and physiological effects of these agents which occur almost immediately (e.g., MAO inhibition itself), but is temporally associated with certain other physiological changes which also only occur after several weeks of treatment (e.g., cardiovascular system changes and alterations in the response to noradrenergic system challenge tests). As the time course and nature of these alterations in patients correspond closely to the time course of certain explicitly-demonstrated brain noradrenergic receptor changes in animals observed with the same drugs in similar doses in our laboratory studies, these studies provide substantial new support for the hypotheses that slowly-developing brain neurotransmitter system adaptational alterations in noradrenergic receptor numbers may be closely involved in the mechanism of action of antidepressant drugs.

Proposed Course:

We plan to extend these studies and thus test the generalizability of the receptor adaptation hypothesis by examining some other clinically effective tricyclic antidepressants using the same pharmacologic challenge paradigms in patients. In addition, we are doing pilot studies of challenge agents for other receptor functions in the noradrenergic system as well as for receptors in other neurotransmitter systems, e.g., the serotonergic system. We will, of course, continue similar studies in patient subgroups during their baseline, off-drug periods for comparison with controls to evaluate other suggestions of abnormalities in specific neurotransmitter systems which may be present in patients with affective disorders--again in parallel with required animal studies.

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Lake, C.R., Pickar, D., Ziegler, M.G., Lipper, S., Slater, S., and Murphy, D.L.: Elevated plasma norepinephrine in patients with major affective disorders. Am. J. Psychiatry, in press.

Siever, L.J., Uhde, T.W., and Murphy, D.L.: Possible subsensitization of α -adrenergic receptors by chronic monoamine oxidase inhibitor treatment in psychiatric patients. Psychiatry Res., in press.

Siever, L.J., Guttmacher, L.B., and Murphy, D.L.: Serotonergic receptors: Evaluation of their possible role in the affective disorders. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorder. Baltimore, Williams and Wilkins Co., in press.

Cohen, R.M., Pickar, D., Garnett, D., Lipper, S., Gillin, J.C., and Murphy, D.L.: REM sleep suppression induced by selective MAO inhibitors. Psychopharmacology, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00327-09 CN |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Dominance in the Marriages of Affective Disorder Patients | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> PI: Carol F. Hoover Social Worker CN NIMH </div> | | |
| COOPERATING UNITS (if any) Department of Psychiatry, Thomas Jefferson University, Philadelphia, PA (R. G. Fitzgerald, M.D.) | | |
| LAB/BRANCH Clinical Neuropharmacology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: <div style="text-align: center;">0.3</div> | PROFESSIONAL: <div style="text-align: center;">0.2</div> | OTHER: <div style="text-align: center;">0.1</div> |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>primary affective</u> disorders were studied on the dimension of <u>marital dominance</u> . Spouses and patients were compared with each other and with couples from the community, using the Conflict in Marriage Scale (CIMS). Patients acknowledged, more often than their spouses, that the partner made final decisions in circumstances of disagreement on family matters. Women patients could be effectively discriminated from women spouses and from community women on this issue. Affectively ill women saw themselves as yielding, after disagreement, to the decision of husbands who seldom changed an opinion. Their male spouses seemed oblivious to these perceptions. This investigation has now been completed; the project is therefore discontinued. | | |

Project Description:

Objectives: This study grew out of an earlier investigation of marital conflict. The new research explored whether the heightened marital conflict of patients with primary affective disorders, compared to married pairs from the community, would be accompanied by a characteristic dominance-dependence relationship between spouses.

Methods Employed:

Items of an agree-disagree card sort, the Conflict in Marriage Scale, were administered to 42 husband-wife pairs, one person of each couple hospitalized for affective illness. These self-reports were then compared to those of 30 married couples from the community, in terms of relative dominance in situations of marital disagreement and decision making.

Major Findings:

Patients acknowledged, more often than their spouses, that the partner made final decisions in circumstances of disagreement on family matters. Women patients could be effectively discriminated from women spouses and from community women on this issue. Affectively ill women saw themselves as yielding, after disagreement, to the decision of husbands who seldom changed an opinion. Their male spouses seemed oblivious to these perceptions.

Significance to Biomedical Research and the Program of the Institute:

An evaluation of the family setting has been important to completing the investigational picture of primary affective disorder, whose biochemical elements have already been extensively studied in the Branch.

Proposed Course:

This investigation has now been completed; the project is therefore discontinued.

Publications:

Hoover, C.F., and Fitzgerald, R.G.: Dominance in the marriages of affective patients. J. Nerv. Ment. Dis. 169: 624-628, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00328-07 CN | | | | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Monoamine Oxidase Activity and Behavior</p> | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Dennis L. Murphy</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">CN NIMH</td> </tr> <tr> <td>OTHER: Robert M. Cohen</td> <td>Staff Physician</td> <td>CN NIMH</td> </tr> <tr> <td>David Pickar</td> <td>Section Chief</td> <td>NE NIMH</td> </tr> <tr> <td>Paul J. Marangos</td> <td>Unit Chief</td> <td>CP NIMH</td> </tr> <tr> <td>Monte S. Buchsbaum</td> <td>Section Chief</td> <td>BP NIMH</td> </tr> </table> | | | PI: Dennis L. Murphy | Chief | CN NIMH | OTHER: Robert M. Cohen | Staff Physician | CN NIMH | David Pickar | Section Chief | NE NIMH | Paul J. Marangos | Unit Chief | CP NIMH | Monte S. Buchsbaum | Section Chief | BP NIMH |
| PI: Dennis L. Murphy | Chief | CN NIMH | | | | | | | | | | | | | | | |
| OTHER: Robert M. Cohen | Staff Physician | CN NIMH | | | | | | | | | | | | | | | |
| David Pickar | Section Chief | NE NIMH | | | | | | | | | | | | | | | |
| Paul J. Marangos | Unit Chief | CP NIMH | | | | | | | | | | | | | | | |
| Monte S. Buchsbaum | Section Chief | BP NIMH | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) R.D. Coursey, University of Maryland; I.C. Campbell, Maudsley Hospital, Dept. of Psychiatry, London, England; L.F. Major, SUNY, Binghamton, NY. | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Clinical Neuropharmacology Branch</p> | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">1.1</td> <td style="text-align: center;">0.4</td> <td style="text-align: center;">0.7</td> </tr> </table> | | | TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | 1.1 | 0.4 | 0.7 | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | |
| 1.1 | 0.4 | 0.7 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Individuals identified originally as constituting a group with low platelet <u>monoamine oxidase</u> (MAO) activity were contacted again two years later to evalu- ate whether personal and family differences from controls with normal MAO activity might persist. Upon follow-up, the low MAO males, who had originally shown a higher incidence of varied forms of psychopathology, continued to show differences from controls, principally in having more job instability, poorer school performance, and more medical problems; while they did not develop more new mental health problems than did controls, they reported more mental health problems in their families, especially depression, alcoholism and suicide attempts--findings in keeping with other suggestions that low MAO activity might represent a risk factor or familial marker for certain types of psychopathology. In laboratory studies examining substrates for the MAO subtypes, norepinephrine was found to be a less selective substrate <u>in vitro</u> for MAO type A than sug- gested by previous studies; this was especially so in primates, including man, in keeping with other data indicating that in man compared to rodents, MAO-B plays a larger functional role in the metabolic degradation of some important <u>neurotransmitter substrates such as dopamine and norepinephrine.</u> | | | | | | | | | | | | | | | | | |

Project Description:

This project is investigating possible relationships between monoamine oxidase (MAO) activity and behavior. Because individual differences in MAO activity are most readily studied in man using blood platelets as sources of the enzyme, the project also had as an objective the study of factors which affect platelet MAO activity and which may be relevant to the interpretations of differences in MAO activity found in psychiatric patient subgroups. The study of the mechanisms of action of drugs which inhibit MAO activity, and the consequences of MAO inhibition on amine neurotransmitter metabolism and other aspects of brain function are additional objectives.

Methods Employed:

Tissues obtained for the study of monoamine oxidase characteristics include human and non-human primate platelets, as well as brain, liver and other mammalian tissues. Drs. Coursey, Buchsbaum, and Major have obtained samples from normals and alcoholic subjects. Several different MAO assay procedures have been adapted or developed in this laboratory for the study of enzyme activity in platelets and other tissues.

Major Findings:

A series of studies have shown that monoamine oxidase (MAO) activity in human platelets is essentially identical in properties to MAO type B measured in other human tissues including brain. According to twin and family investigations in normals, platelet MAO activity is predominantly under genetic control. Differences in MAO activity between various psychiatric patient groups and controls have been frequently reported, with alcoholic and bipolar I affective disorder patients most often found to have reduced platelet MAO activity, while in some studies patients with secondary or non-endogenous unipolar affective disorders have been found to have higher MAO activity. Chronic schizophrenic patients also have reduced MAO activity, although neuroleptic treatment may contributed to the observed platelet MAO changes in this population. Family members of alcoholic and bipolar affective disorder patients also have MAO values and/or incidences of these disorders which are different from controls, a finding which has been interpreted as suggesting that altered MAO activity may not be just a marker for these disorders, but might represent a familial or even genetic vulnerability factor or risk factor. However, inconsistencies across the different reports in the literature and various ways in which exogenous factors (including non-specific stress factors and drug treatment) may have affected the results limit the conclusions which can be drawn from many of the present investigations in patients. One of our investigators in the past year, however, clarified that although alcohol withdrawal transiently alters circulatory platelet numbers and platelet MAO activity, the differences observed among alcoholic patients, relatives of alcoholics, and controls are not an artifact of alcohol ingestion. A comprehensive review of platelet MAO activity measurements in various patient groups and the limitations of this study approach was published in the last year.

Our current studies evaluating the relationships between reduced platelet MAO and psychologic characteristics in normals included a two-year follow-up investigation of low MAO subjects from our previous biological high risk study.

Thirty-three community college students identified solely on the basis of low platelet MAO activity and 30 controls from the previously screened population of 375 individuals were interviewed for a second time two years after their first interview. The low MAO males who had originally shown a higher incidence of both personal and familial psychopathology continued to exhibit differences from controls. The low MAO males reported more job instability, and had also fallen about half a year behind than high MAO counterparts in school. No new differences in other aspects of social status or psychosocial problems had developed in these subjects, although they tended to have developed more major or minor medical problems. They also reported more mental health problems in their families, especially depression, alcoholism and suicide attempts.

In laboratory studies of the MAO-A and MAO-B subtypes, neither enzyme form demonstrated a high degree of colocalization with an enzyme marker for neurons, neuron-specific enolase, suggesting that both MAO forms may be present in glia as well as in neurons, and that the close association in the periphery between MAO type A and sympathetic neurons may prove to be a special case for this neuronal system. In a related investigation, *in vitro* studies of MAO activity in a number of tissues from different species using the irreversible MAO-A inhibitor clorgyline demonstrated that serotonin is a more selective substrate for MAO-A than is norepinephrine. These differences are greater in humans and other primates than in rodents. Serotonin also has a smaller apparent K_m for MAO-A than α -norepinephrine and is deaminated more readily by MAO in 10 of the 11 tissues studied. Correspondingly, the MAO-B in human platelets deaminates α -norepinephrine more readily than serotonin and has a 3-fold smaller K_m for α -norepinephrine than serotonin. These data provide the clearest evidence in the literature thus far indicating that the preferential deamination of norepinephrine by MAO-A is more evident in the peripheral than central nervous system. These findings also suggest that further examination in clinical situations is needed to determine whether selective MAO-A inhibitors like clorgyline might have relatively greater effects on serotonin than norepinephrine.

Significance to Biomedical Research and the Program of the Institute:

Difficulties in interpreting the significance of associations between platelet MAO activity and psychiatric diagnoses because of possible influences of the patient's clinical state and the lingering effects of drug treatment on both the MAO enzyme and on platelet production led to the development of the biological high risk strategy. This strategy proved successful in its first application to MAO activity differences in normals, and in this year's work the original results were verified in a two-year follow-up study. The biological high risk strategy has since been applied to investigate other putative biological markers or vulnerability factors for psychopathology besides MAO activity. Interest in the possible significance of this enzyme for psychopathology and, of course, in the characteristics of the enzyme in regard to the mode of action of MAO-inhibiting antidepressants led to additional studies delineating the activity of the two forms of MAO in degrading specific neurotransmitter amines, exemplified this year in our studies demonstrating norepinephrine to be metabolized by both MAO-A and MAO-B.

Proposed Course:

Our group's major current investment in studies of monoamine oxidase are related to investigations of inhibitors of the enzyme with antidepressant properties, as summarized in our other project report, Z01 MH 00326-09. We have, with the follow-up study, concluded our work with platelet MAO activity as a marker for psychopathology, and plan to use the platelet MAO assay only as a means to monitor the degree of MAO inhibition produced by MAO-inhibiting antidepressants in individual patients. Our in vitro studies of MAO as an enzyme are also more closely related to our work with MAO-inhibitors as antidepressants, and so we are terminating this project with this year's final report.

Publications:

Murphy, D.L., Pickar, D., Jimerson, D., Cohen, R.M., Garrick, N.A., Karoum, F., and Wyatt, R.J.: Biochemical indices of the effects of selective MAO inhibitors (clorgyline, pargyline and deprenyl) in man. In Usdin, E., Dahl, S., Gram, L.F., and Lingjaerde, O. (Eds.): Clinical Pharmacology in Psychiatry. London, MacMillan Press, 1981, pp. 307-316.

Campbell, I.C., Marangos, P.J., Murphy, D.L., and Pearse, A.G.E.: Neuron specific enolase (NSE) in human blood platelets: Implications for the neuronal model. In Angrist, B., Burrows, G.D., Lader, M., Lingjaerde, O., Sedvall, G., and Wheatley, D. (Eds.): Recent Advances in Neuropsychopharmacology. Oxford, Pergamon Press, 1981, pp. 203-211.

Major, L.F., Goyer, P.F., and Murphy, D.L.: Changes in platelet monoamine oxidase activity during abstinence. J. Stud. Alcohol 42: 1052-1057, 1981.

Coursey, R.D., Buchsbaum, M.S., and Murphy, D.L.: Two-year follow-up of subjects and their families defined as at risk on the basis of platelet MAO activities. Neuropsychobiology 8: 51-56, 1982.

Garrick, N.A., and Murphy, D.L.: Differences in the preferential deamination of α -norepinephrine, dopamine and serotonin by monoamine oxidases in rodent and primate brain. In Usdin, E., Weiner, N., and Creveling, C. (Eds.): Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects. London, MacMillan Press, 1981, pp. 517-526.

Murphy, D.L., Coursey, R.D., Haenel, T., Aloï, J.A., and Buchsbaum, M.S.: Platelet monoamine oxidase as a biological marker in the affective disorders and alcoholism. In Usdin, E., and Hanin, I. (Eds.): Biological Markers in Psychiatry and Neurology. New York, Pergamon Press, 1982, pp. 123-136.

Coursey, R.D., Buchsbaum, M.S., and Murphy, D.L.: Monoamine oxidase activity and the longitudinal biological high risk approach to schizophrenia and affective illnesses. In Mednick, S.A., and Harway, M. (Eds.): Longitudinal Research in the United States. Boston, Martinus Nijhoff, in press.

Garrick, N.A., and Murphy, D.L.: Monoamine oxidase type A: Differences in selectivity towards L-norepinephrine compared to serotonin. Biochem. Pharmacol., in press.

Campbell, I.C., Marangos, P.J., Parma, A., Garrick, N.A., and Murphy, D.L.: The localization of monoamine oxidase types A and B in primate brains relative to neuron-specific and non-neuronal enolases. Neurochem. Res., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00329-07 CN | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Platelets as Models for the Study of Neurotransmitter Function | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Jonathan L. Costa</td> <td style="width: 30%;">Staff Physician</td> <td style="width: 20%;">CNB NIMH</td> </tr> <tr> <td rowspan="8">COLLAB:</td> <td>R. Blumenthal</td> <td>Section Chief</td> <td>LTB NCI</td> </tr> <tr> <td>C. R. Creveling</td> <td>Biologist</td> <td>LBC NIADDK</td> </tr> <tr> <td>E. D. Eanes</td> <td>Section Chief</td> <td>LBS NIDR</td> </tr> <tr> <td>J. I. Gallin</td> <td>Section Chief</td> <td>LCI NIAID</td> </tr> <tr> <td>K. L. Kirk</td> <td>Chemist</td> <td>LC NIADDK</td> </tr> <tr> <td>J. M. Launay</td> <td>Guest Worker</td> <td>CNB NIMH</td> </tr> <tr> <td>A. J. Robinson</td> <td>Guest Worker</td> <td>CM NHLBI</td> </tr> <tr> <td>E. L. Veech</td> <td>Laboratory Chief</td> <td>LM NIAAA</td> </tr> <tr> <td></td> <td>E. C. Weinbach</td> <td>Section Chief</td> <td>LPD NIAID</td> </tr> </table> | | | PI: | Jonathan L. Costa | Staff Physician | CNB NIMH | COLLAB: | R. Blumenthal | Section Chief | LTB NCI | C. R. Creveling | Biologist | LBC NIADDK | E. D. Eanes | Section Chief | LBS NIDR | J. I. Gallin | Section Chief | LCI NIAID | K. L. Kirk | Chemist | LC NIADDK | J. M. Launay | Guest Worker | CNB NIMH | A. J. Robinson | Guest Worker | CM NHLBI | E. L. Veech | Laboratory Chief | LM NIAAA | | E. C. Weinbach | Section Chief | LPD NIAID |
| PI: | Jonathan L. Costa | Staff Physician | CNB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COLLAB: | R. Blumenthal | Section Chief | LTB NCI | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | J. M. Launay | Guest Worker | CNB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | A. J. Robinson | Guest Worker | CM NHLBI | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | E. L. Veech | Laboratory Chief | LM NIAAA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | E. C. Weinbach | Section Chief | LPD NIAID | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) L. Corash, Hematology Unit Medical Center, San Francisco, CA; C.R. Valeri, Naval Blood Research Laboratory, Boston, MA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Neuropharmacology Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">3.0</td> <td style="text-align: center;">2.5</td> <td style="text-align: center;">0.5</td> </tr> </table> | | | TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | 3.0 | 2.5 | 0.5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> Platelets from humans and other species have been used to continue the study of <u>amine uptake</u>, <u>amine metabolism</u>, <u>amine storage</u>, and <u>amine release</u>. Since these processes have been shown to possess a complex interrelationship in intact platelets, similar processes in <u>model systems</u>, <u>mitochondria</u>, <u>nerve microsacs</u>, and <u>microvessels</u> have been examined to explore more completely the nature of the individual components. In addition, some of the new techniques developed utilizing normal platelets have been applied to the study of platelets from patients with disorders of <u>amine storage</u>. The results provide a fundamental basis for understanding the normal and pathological regulation of aminergic systems in platelets and brain tissue, and suggest strategies for pharmacological alteration of this regulation. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: The principal object of this work is to explore mechanisms for amine metabolism, uptake, storage, and release. Many of the studies utilize platelets from humans and other species, but isolated mitochondria, isolated microvessels, and nerve microsacs are also examined. In addition, the project studies possible mechanisms for the action of psychoactive drugs on aminergic systems.

Studies Implemented: (1) Evaluation of the transport of calcium in a Pressman cell (bulk) system; (2) preparation of analogues of mitochondrial dense granules; (3) evaluation of the subcellular distribution of indium-111 in human platelets; (4) effects *in vitro* and *in vivo* of psychoactive drugs on mitochondrial oxidative phosphorylation; (5) testing of various possible mechanisms for vesicular amine storage in human platelets; (6) examination of amine metabolism in platelets from patients with low platelet serotonin; (7) examination of the role of phenolsulfotransferase in serotonin disposition in platelets, microvessels, and nerve microsacs; (8) comparison in human platelets of the uptake of a wide spectrum of indole derivatives and drugs with their effects on the uptake and release of serotonin.

Methods Employed:

1. General Preparative Procedures: Platelets are isolated from normal volunteers or from patients by differential centrifugation or platelet-phoresis. Mitochondria are prepared from rat liver or brain, or from bovine heart, by homogenization and differential centrifugation.

2. Preparation and Analysis of Models for Amine Storage Vesicles and Mitochondrial Granules. Utilizing an apparatus designed to form precipitates under defined conditions, solid precipitates of varying composition are prepared. The precipitates are analyzed for metal and phosphate-moiety content, and manipulated in various ways to explore the factors important in their formation and dissolution, and in the binding of amines.

3. Evaluation of Calcium Transport in Bulk Systems. A modified Pressman cell is used to study ionophore-associated fluxes of calcium between aqueous compartments. Transport kinetics and the influence of various ions on the process are studied, and any precipitate formed is studied by chemical analysis and X-ray diffraction.

4. Exploration of a Possible Mitochondrial Role in the Action of Psychoactive Drugs. Body temperature and behavior are monitored in rats following injections of various drugs. Oxygen consumption of homogenates and isolated mitochondria is measured polarographically with an oxygen electrode before and after addition of psychoactive drugs. Activity of various mitochondrial enzymes is measured spectrophotometrically or by fluorescence spectroscopy. Mitoplasts free of outer membranes are prepared from beef heart mitochondria and studied polarographically and spectrophotometrically.

5. Subcellular Distribution of Radiolabels in Human Platelets. Platelets labelled with various compounds are homogenized and fractionated utilizing sucrose or Metrizamide density gradients. Fractions are analyzed for radioactivity, by electron microscopy, and following equilibrium dialysis. The effects of various manipulations of intact platelets on the subcellular distribution of radioactivity is also evaluated.

6. Examinations of Mechanisms of Amine Storage in Human Platelets. Synthetic analogues of the core of human-platelet storage vesicles are prepared in aqueous and organic solvents, and the binding of various amines and psychoactive substances is evaluated by Scatchard analysis. Washed human platelets are loaded with amines and then incubated with substances known to affect pH gradients or to inhibit uptake into vesicles.

7. Amine Metabolism in Serotonin Deficient Platelets. Platelets from patients with a low platelet serotonin content are studied utilizing radiolabelled tracers, thin layer chromatography, radioenzymology, and electron microscopy.

8. Assessment of Phenosulfotransferase in Various Tissues. Phenolsulfotransferase activity is assayed utilizing alveolysin toxin to break cells and biogenic amines as substrates, and sulfated products are quantitated by thin layer chromatography. These techniques are applied to various amine-sequesting tissues before and after addition of psychoactive drugs.

9. Parameters Affecting Serotonin Uptake and Release by Human Platelets. An accurate and reproducible method is used for quantitating uptake of radiolabelled serotonin, and the uptake system is perturbed by changes in the composition of the extracellular medium or by the addition of a wide variety of indole derivatives. Uptake of various compounds is measured fluorometrically, or following the radioenzymatic or chemical synthesis of labelled compounds. Release of vesicular serotonin is monitored fluorometrically and by liquid scintillation counting after addition of various compounds or psychoactive drugs.

Major Findings:

1. Analysis of Models for Amine Storage Vesicles and Mitochondrial Granules. Precipitates similar in composition to platelet dense bodies form equally well in the presence or absence of magnesium, and the solids themselves appear to favor calcium incorporation at the expense of magnesium. Although high concentrations of both lithium and serotonin accumulate in platelet dense bodies, both substances show no greater incorporation into the solid than potassium or sodium ions. The observed accumulation of lithium thus cannot be attributed to competition with calcium for binding to nucleotides in the dense body core. Study of the process of accumulation of pyrophosphate in mitochondria of butyrate-treated rats suggests that the resulting mitochondrial granules are not composed exclusively of calcium pyrophosphate, but include also appreciable amounts of adenine nucleotides and orthophosphate. Nevertheless, a simple precipitation reaction in the mitochondrial matrix does not appear to account for the cation and anion changes observed following butyrate administration.

2. Calcium Transport in Bulk Systems. Ionophores can mediate calcium/potassium and calcium/proton countertransport through an organic phase between aqueous compartments, and the transport can be accelerated by the presence of phosphate groups in one compartment. The resultant precipitate is amorphous in composition, as are platelet dense bodies. A similar mechanism may be responsible for the influx of calcium and possibly lithium ions into platelet storage vesicles.

3. Possible Mitochondrial Role in the Action of Psychoactive Drugs. Although reserpine is a relatively potent uncoupling agent when added to mitochondria *in vitro*, it does not behave as a "classical" uncoupler (i.e., a protonophore) in terms of its action in mitochondrial ATPase, its effects on mitoplasts, and its lack of synergistic effect in the presence of other uncoupling agents. Several other psychoactive substances also uncouple respiration in isolated liver and brain mitochondria. Nevertheless, the pharmacological actions of these drugs appear not to be related to their uncoupling activity, since the correlation is poor between uncoupling potency and action as a psychotomimetic, since mitochondria in homogenates of drug-treated rats are not uncoupled, and since the animals exhibit none of the other stigmata seen following injection of classical uncoupling agents. Chronic injections of pargyline, in contrast, do appear to induce the uncoupled state in mitochondria both *in vivo* and *in vitro*.

4. Subcellular Distribution of Radiolabels in Human Platelets. Both indium-111 and chromium-51, isotopes commonly used to tag platelets for the evaluation of platelet turnover and lifespan, appear to be sequestered in the platelet cytoplasm, loosely bound to platelet proteins. Although the uptake of indium-111 has been reported to occur by a diffusional process, pre-treatment of cells with metabolic poisons decreases the uptake and retention of the label. Work is currently underway to develop radioligands for other subcellular sites in platelets (i.e., storage vesicles).

5. Mechanisms of Amine Storage in Human Platelets. Experiments have been designed to test a series of hypotheses about the mechanism of amine storage in the dense bodies of human platelets. Participation in a macromolecular aggregate, binding to the core constituents of the dense bodies, and the continual operation of a "pump-leak" system do not appear to play appreciable roles in serotonin storage. Although the existence of a vesicle-cytoplasm pH gradient may be important for the storage of some amines such as quinacrine, it does not seem to be a prerequisite for serotonin or dopamine storage. The model most consistent with the observed data suggests dramatically restricted permeability of the dense body membrane to these amines following intra-vesicular accumulation by a carrier-mediated mechanism.

6. Amine Metabolism in Serotonin-Deficient Platelets. Platelets from patients with classical storage pool deficiency (Chediak-Higashi syndrome) contain reduced numbers of storage sites (dense bodies) and an apparently increased cytoplasmic serotonin content (which is serotonin and not a metabolite). Platelets from another patient with a low serotonin content have normal numbers of dense bodies and an apparent increase in cytoplasmic serotonin stores. The dense bodies thus lack a normal storage capacity, which may be related to an altered glutathione metabolism in these platelets.

7. Phenolsulfotransferase in Various Tissues. Evaluation of the o-sulfation of serotonin and its relationship to serotonin uptake and metabolism has been facilitated by development of an enzyme assay procedure utilizing alveolysin toxin and of a thin layer-chromatography system for quantitating serotonin-o-sulfate. The enzyme activity in human platelets is unique in its substrate preferences and sensitivity to various inhibitors. Under normal circumstances, only 1-2% of the total serotonin in the cell exists in the o-sulfated form, but this percentage can be increased by treatment with certain drugs. Sulfation of the 4,6-difluoro analogue of serotonin may play a role in its anomalous compartmentation in platelets. Phenolsulfotransferase in nerve microsacs and microvessels has similar substrate preferences but unique responses to inhibitory compounds. In both tissues, the percentage of o-sulfated serotonin present can be increased following the addition of certain drugs, and some of the o-sulfated material may be released from the cell. In microvessels, o-sulfation of serotonin may play a role in the serotonin uptake mechanism.

8. Serotonin Uptake and Release by Human Platelets. The kinetics of serotonin uptake has proven to be exquisitely sensitive to the isolation procedure for platelets, the salt form of the serotonin used, and the sugar and ion content of the extracellular medium. An interesting and complex series of interrelationships exists between the steric and electronic structure of various indolic compounds as they affect serotonin uptake, are themselves taken up, and themselves cause release of endogenous vesicular serotonin. Certain psychoactive substances with indole-like groupings can add to or antagonize the observed effects.

Significance to Biomedical Research and the Program of the Institute:

Progress continues to be made in elucidating the cellular mechanisms responsible for vesicular and cytoplasmic amine storage, and the nature of the plasma-membrane uptake system for indolic compounds, in human platelets. As more is discovered, however, it becomes more clear that all the systems are more complex than previously believed. In addition, a similar degree of complexity appears to exist in other amine-storing tissues such as nerve microsacs and microvessels. This work lays the foundation for an improved understanding of aminergic systems in platelets and brain tissue, thus extending our capacity to understand pathological alterations and suggesting strategies for successful pharmacological intervention.

Proposed Course:

Continue the work. Areas needing continued careful scrutiny are the extent of amine permeability of the vesicular membrane, the effects of psychoactive drugs on phenolsulfotransferase activity, the relationship between phenolsulfotransferase action and serotonin uptake, and the design of new psychoactive agents based on the principles outlined here.

Publications:

Costa, J.L., Murphy, D.L., and Stark, H.: Applicability of models for carrier-mediated serotonin transport to pools of serotonin in intact human platelets. J. Physiol. 316: 153-161, 1981.

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Costa, J.L., Kirk, K.L., and Stark, H.: Utility of ten-second uptake periods for kinetic studies of serotonin uptake by intact platelets. Res. Comm. Pathol. Pharmacol. 33: 547-558, 1981.

Costa, J.L., Eanes, E.D., Fay, D.D., and Hailer, A.W.: Preparation and characterization of synthetic models for the dense bodies of human platelets. Cell Calcium 2: 459-472, 1981.

Costa, J.L., Kirk, K.L., and Stark, H.: Uptake of 6-fluoro-5-hydroxytryptamine and 4,6-difluoro-5-hydroxytryptamine into releasable and non-releasable compartments of human platelets. Br. J. Pharmacol. 75: 237-242, 1982.

Eanes, E.D., and Costa, J.L.: X-537A ionophore-mediated calcium transport and calcium phosphate formation in Pressman cells. Calcified Tissue International. in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00330-04 CN | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Use of Electron and Photon Imaging Techniques to Study Aminergic Systems</p> | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Jonathan L. Costa</td> <td style="width: 33%;">Staff Physician</td> <td style="width: 33%;">CN NIMH</td> </tr> <tr> <td>OTHER: R.D. Leapman</td> <td>Physicist</td> <td>MAS BEIB</td> </tr> <tr> <td>E.D. Eanes</td> <td>Section Chief</td> <td>LBS NIDR</td> </tr> </table> | | | PI: Jonathan L. Costa | Staff Physician | CN NIMH | OTHER: R.D. Leapman | Physicist | MAS BEIB | E.D. Eanes | Section Chief | LBS NIDR |
| PI: Jonathan L. Costa | Staff Physician | CN NIMH | | | | | | | | | |
| OTHER: R.D. Leapman | Physicist | MAS BEIB | | | | | | | | | |
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| COOPERATING UNITS (if any) G.C. Chapline, Lawrence Livermore Lab, Livermore, CA; R. Feder and D. Sayre, IBM Res. Labs, Yorktown Hts., NY; J. Kirz, SUNY, Stonybrook, NY; J. Pearlman, Maxwell Labs, San Diego, CA; J.D. Solem, Los Alamos Labs, NM; Brookhaven National Labs, Upton, Long Island, NY | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Clinical Neuropharmacology Branch</p> | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) <p> New techniques are being developed which employ <u>electrons</u> and long-wavelength <u>x-rays (photons)</u> to study subcellular architecture, and the chemical and physical environment of elements on a molecular scale. These include <u>electron energy-loss spectroscopy</u>, <u>contact x-ray microscopy</u>, <u>scanning x-ray microscopy</u>, and <u>x-ray holography</u>. It seems possible with both photons and electrons to pinpoint the location of relatively small numbers of tracer molecules inside cells. </p> | | | | | | | | | | | |

Project Description:

Objectives: The work emphasizes the development and application of techniques for studying the structural parameters associated with cellular function. The methods developed are subsequently used to examine the macro- and micro-structure of platelets and other systems known to store amines.

Studies Implemented: (1) Critical examination of flash x-ray sources with respect to suitability for laboratory use; (2) development of instrumentation for a scanning x-ray microscope with the capacity for elemental analysis; (3) exploration of the radiation sensitivity of various fluorine-containing compounds of interest; (4) electron and x-ray diffraction studies of tissues from patients with disorders of calcification; (5) outline of experimental protocols required for imaging with an x-ray laser, and preliminary study of specimens; (6) examination of the feasibility of diffraction studies using long-wavelength x-rays; (7) contact x-ray microscopy of test specimens, including bacteria, nerve microsacs, and platelets.

Methods Employed:

1. General Preparative Procedures. Platelet whole mounts are prepared by rapid air drying, and platelet thin sections from conventionally fixed and dehydrated material. Whole mounts of bacteria, nerve microsacs, and model systems are prepared in a similar manner. For studies of radiation damage, compounds are dissolved in ethanol and dried onto grids by blotting. Other tissue specimens are mounted for wet examination, or fixed and thin-sectioned, or incinerated and placed on grids.

2. Evaluation of Flash X-Ray Sources. Test specimens are mounted on electron microscope grids and placed in contact with the recording medium (x-ray resist). Various combinations of filters are placed between the source and the specimen, the source is fired, and the exposed resist developed and studied in the light microscope.

3. Implementation of a Scanning X-Ray Microscope. X-ray diffraction and focussing devices are designed after consideration of the requirements for elemental analysis and imaging. Specimens are prepared and studied in the light microscope and the x-ray microscope prototype to determine optimum thickness.

4. Radiation Sensitivity Studies. Electron energy-loss spectra are collected utilizing calibrated radiation intensities, and the peak integrals of elements of interest are quantitated.

5. Electron and X-Ray Diffraction Studies. Diffraction patterns are obtained from various tissue preparations. Areas of interest are also analyzed by electron microprobe techniques.

6. Development of an X-Ray Laser. Plans are made for measurement of positions of absorption edges and resonance lines of compounds and specimens of interest, and appropriate specimens are prepared. Nanosecond exposure characteristics of x-ray resist are evaluated utilizing a flash x-ray source.

7. Long-Wavelength X-Ray Diffraction. Calculations are made to estimate sample thicknesses, exposure times, and camera distances. Specimens with appropriate characteristics to test the diffraction system are prepared and examined by contact x-ray microscopy.

8. Contact X-Ray Microscopy. Contact images are obtained utilizing a specially-designed source of carbon K_{α} x-rays to expose polymethyl-methacrylate polymer. The developed x-ray images are coated and studied in the transmission electron microscope.

Major Findings:

1. Flash X-Ray Microscopy. The most promising configuration for flash x-ray microscopy appears to be a plasma-pinch device with a 10-20 nanosecond pulse time. In order to obtain debris-free resists with sufficiently high resolution, some modification of the existing instrument will be required. Another possibility is a laser-excited source, and work continues on the evaluation of this instrument. Resist exposures with a 10-20 nanosecond pulse time appear to be adequate.

2. Scanning X-Ray Microscopy. A diffraction grating with appropriate wavelength resolution and reflectance in the wavelength region spanning elements of interest (carbon through fluorine) has been designed, fabricated, and installed at the Brookhaven Synchrotron. In addition, a focussing zone plate has been fabricated, and a specimen stage has been constructed. Techniques for providing specimens which appear to have suitable thickness for x-ray microscopy and elemental analysis have been developed.

3. Radiation Sensitivity Studies. Fluorine atoms covalently bonded to an aromatic ring appear to be much less sensitive to electron-induced loss than expected. Some of the ring-fluorinated biogenic amines, for example, can tolerate radiation doses sufficient to permit detection of relatively small amounts of fluorine.

4. Electron X-Ray Diffraction Studies. Electron microprobe and diffraction studies have identified hydroxylapatite as the principal component in cutaneous lesions in a patient with an obscure genetic disorder.

5. Development of an X-Ray Laser. Utilizing the resonance lines of nitrogen and fluorine, it should be possible to obtain x-ray holograms of cellular structures containing these elements with a resolution of 50-100 Å. Polymethyl-methacrylate resist appears to provide resolution and sensitivity adequate for a recording medium, and can subsequently be examined by conventional electron microscopy.

6. Long-Wavelength X-Ray Diffraction. Cellular structures as small as individual strands of chromatin, a single mitochondrion, or a single amine storage vesicle appear sufficiently opaque to long-wavelength x-rays to produce useful diffraction patterns. Appropriate model specimens are available to check the diffraction setup at the Brookhaven Synchrotron once it becomes operational.

7. Contact X-Ray Microscopy. The photon-absorbent cytoplasmic network seen in human platelets may represent a cytoskeletal structure, but does not seem to be composed exclusively of actin filaments. Similar networks are seen in bacteria and in nerve microsacs, but not in red blood cells. Photon diffraction does not seem to provide an explanation for the widespread occurrence of cytoskeletal structures, since crystals of calcium pyrophosphate of appropriate size do not produce similar images. The three-dimensional relationships of amine storage vesicles to the cytoskeletal network of platelets can be explored in detail by making stereo x-ray replicas of human platelets.

Significance to Biomedical Research and the Program of the Institute:

The long-term goal of this project is to determine the anatomical location of various pools of amines in human platelets and to explore the chemical and geometrical environments surrounding amine molecules inside storage vesicles. Accomplishment of this goal will require implementation of new biophysical techniques involving photons and electrons, and much of the work done this year has been directed toward technical development. Once the appropriate methods are available, however, they will provide extremely powerful and important tools with which to study a wide variety of cellular functions, including those occurring in hydrated material. During the developmental stage of the work, contact x-ray microscopy using a relatively low-powered photon source has provided useful information about the proper methods of specimen preparation, as well as new insights into the complexity of cellular architecture in human platelets and nerve endings.

Proposed Course:

Continue the work, emphasizing subcellular localization of fluorinated amines in various aminergic tissues.

Publications:

Costa, J.L., Fay, D.D., and McGill, M.: Electron probe microanalysis of calcium and phosphorus in dense bodies isolated from human platelets. Throm. Res. 22: 399-405, 1981.

Feder, R., Costa, J.L., Chaudhari, P., and Sayre, D.: Improved detail in biological soft x-ray microscopy: Study of blood platelets. Science 212: 1398-1400, 1981.

Costa, J.L., Smith, M.A., Tanaka, Y., and Cushing, R.J.: Phosphorus and divalent cations in dog, rabbit, and human platelet dense bodies as deduced from electron microprobe studies of air-dried whole mounts. Res. Commun. Chem. Pathol. Pharmacol. 32: 137-145, 1981.

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| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Use of Nuclear Magnetic Resonance to Study Aminergic Systems</p> | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Jonathan L. Costa</td> <td style="width: 30%;">Staff Physician</td> <td style="width: 20%;">CN NIMH</td> </tr> <tr> <td>OTHER:</td> <td>K. L. Kirk</td> <td>Chemist</td> <td>LC NIADDK</td> </tr> <tr> <td></td> <td>S. J. Morris</td> <td>Expert Consultant</td> <td>NTS NINCDS</td> </tr> <tr> <td></td> <td>E. Sokoloski</td> <td>Chemist</td> <td>LC NHLBI</td> </tr> <tr> <td></td> <td>E. C. Weinbach</td> <td>Section Chief</td> <td>LPD NIAID</td> </tr> </table> | | | PI: | Jonathan L. Costa | Staff Physician | CN NIMH | OTHER: | K. L. Kirk | Chemist | LC NIADDK | | S. J. Morris | Expert Consultant | NTS NINCDS | | E. Sokoloski | Chemist | LC NHLBI | | E. C. Weinbach | Section Chief | LPD NIAID |
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| COOPERATING UNITS (if any) <p style="text-align: center;">D. Diffley, Medical Student, Johns Hopkins University, Baltimore, MD</p> | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Clinical Neuropharmacology Branch</p> | | | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) <p> The nature of the <u>amine storage complexes</u> in vesicles of <u>platelets</u> from various species has been studied utilizing the technique of <u>nuclear magnetic resonance</u>. The molecular mobility of intra-vesicular <u>serotonin</u> appears to be a unique characteristic of each species, suggesting that wide variation is possible in the nature of the complex. In human platelets, extra-vesicular amine also appears to have characteristic chemical and motional properties. With the use of <u>ring-fluorinated amines</u>, it is now possible to study more critically the nature of vesicular amine storage in <u>nerve microsacs</u>. </p> | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: To use nuclear magnetic resonance (NMR) in conjunction with conventional biochemical techniques to study the dynamic processes governing amine uptake, metabolism, storage, and release in platelets and other amine-storing systems.

Studies Implemented: (1) Examination of the effects of reserpine on various pools of fluorinated serotonin in platelets; (2) exploration of glucose metabolism in platelets and other organisms; (3) comparison of mechanisms of serotonin storage in platelets from humans with those in platelets of other species; (4) study of the motional properties, osmotic sensitivity and macrostructure of chromaffin vesicles; (5) definition of the nature of the storage complex for dopamine in nerve microsacs.

Methods Employed:

(1) General Preparative Procedures. Platelets are isolated from various animal species, normal volunteers, or patients on lithium therapy by differential centrifugation. Eukaryotic cells are grown on chemically-defined media and harvested by a filtration apparatus. Chromaffin vesicles are prepared from bovine adrenal glands by homogenization and differential centrifugation. Nerve microsacs are prepared from guinea pig brains by homogenization and filtration. Cells, microsacs, and isolated vesicles are incubated according to various protocols and prepared for NMR study by extraction with perchloric acid or by resuspension at a high cell density.

(2) NMR Studies. Depending on the labelled compound used, the phosphorus-31, carbon-13, or fluorine-19 nuclei are examined. The linewidths and chemical-shift positions of known compounds are measured, and used as external or internal standards with which to compare similar parameters in the biological material of interest. The linewidth as a function of temperature in the range 4°C-37°C is estimated, and in some cases used to calculate an activation energy and probable correlation time.

Major Findings:

(1) Effects of Reserpine on Serotonin Pools in Platelets. Human platelets loaded with 4,6-difluoroserotonin in the presence of reserpine appear to contain cytoplasmic (extra-vesicular) pools of both difluoroserotonin and a second related compound. Rabbit platelets when incubated under similar conditions, in contrast, show only peaks characteristic of vesicular difluoroserotonin.

(2) Glucose Metabolism. As a test system, the metabolism of C^{13} -labelled glucose by Girardia lamblia has been studied. This organism appears to remove enzymatically the carbon in the one position and release it as carbon dioxide, while a portion of the remainder of the glucose chain is incorporated into lipidic structures. Similar studies should assist in the elucidation of the pathways of platelet metabolism under normal conditions and during the release reaction.

(3) Serotonin Storage Complexes in Platelets from Various Species. In dense bodies of human platelets, both the serotonin ring and the nucleotide phosphate groups appear to be completely immobilized (i.e., in a solid state). They remain in this condition despite the addition of quinacrine to the vesicle. In vesicles of pig platelets, the serotonin ring is entrapped in a gel-like matrix of adenine nucleotides and magnesium, whose relative motional properties vary with temperature. Addition of quinacrine to the pig vesicles increases the molecular mobility of both the serotonin ring and the nucleotide phosphate groups. The serotonin ring in vesicles of rabbit platelets resides in a molecular environment more mobile than that found in vesicles of either pig or human platelets, but still temperature dependent and less mobile than that of chromaffin vesicles.

(4) Osmotic Properties of Chromaffin Vesicles. Under normal circumstances, and at temperatures ranging from 4°C to 37°, amines and nucleotides inside chromaffin vesicles move relatively freely in an essentially aqueous environment. Dehydration of the vesicles by suspension in hyperosmolar sucrose alters the mobility pattern of the nucleotides so that it resembles the gel-like condition found inside the vesicles of pig platelets, (i.e., slow tumbling at 4°C and an increasing rate up to 30°C). In contrast to the nucleotides in pig platelet vesicles, however, the nucleotides inside dehydrated chromaffin vesicles do not appear to increase in mobility as quinacrine is added.

(5) Dopamine Storage Complex in Nerve Microsacs. When allowed to enter nerve microsacs by active transport, 6-fluorodopamine appears to accumulate in a compartment in which it has a significantly restricted mobility at 4°C and an increased mobility as the temperature is raised. This pattern of temperature-dependent molecular motion is also seen for fluorinated dopamine inside vesicles of pig platelets, and suggests a similar type of vesicular storage site in nerve microsacs. This conclusion is supported by the observation that pretreatment with reserpine before 6-fluorodopamine allows the molecule to accumulate in a compartment without any temperature sensitivity or apparent restriction of motion.

Significance to Biomedical Research and the Program of the Institute:

Although the mechanisms responsible for storage of biogenic amines inside vesicles remain unclear, NMR provides a powerful new tool with which to explore the problem. This work began by examining vesicular amine storage in platelets and chromaffin vesicles, both readily accessible amine-storing tissues which are stable under NMR conditions. In human platelets, the levels of cytoplasmic (extra-vesicular) amine can be sufficiently large to produce distinct NMR signals, thus facilitating exploration of the role of these amine pools in amine uptake, metabolism, and release. It has now proved possible to implement similar studies in nerve microsacs, allowing correlation of the properties of the vesicular storage complex for several amine neurotransmitters with those better understood from examination of other tissues.

Proposed Course:

Continue the work, with special emphasis on examining the molecular mobility of the amine side chain in various storage complexes. The work with fluorinated amines in particular sets the stage for possible future clinical applications of NMR imaging systems.

Publications:

Costa, J.L., Dobson, C.M., Kirk, K.L., Poulsen, F.M., Valeri, C.R., and Vecchione, J.J.: Nuclear magnetic resonance studies of amine storage in pig platelets. FEBS Letters (in press).

Costa, J.L., Fay, D.D., Nurnberger, J.I., and Murphy, D.L.: Preferential accumulation of lithium in the dense bodies of human platelets. Biochem. Pharmacol. (in press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00332-04 CN | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Animal Models for the Study of Neuropharmacologic Effects</p> | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> PI: Robert M. Cohen OTHER: Dennis L. Murphy Charanjit S. Aulakh John W. Daly Irwin J. Kopin David Pickar Richard P. Ebstein John F. Tallman </td> <td style="width: 50%; vertical-align: top; border-left: 1px solid black; padding-left: 10px;"> Staff Physician Chief Visiting Fellow Chief Chief Section Chief Visiting Fellow Section Chief </td> <td style="width: 50%; vertical-align: top; border-left: 1px solid black; padding-left: 10px;"> CN NIMH CN NIMH CN NIMH LBC NIADDK LCS NIMH NB NIMH LBC NIADDK NB NIMH </td> </tr> </table> | | | PI: Robert M. Cohen OTHER: Dennis L. Murphy Charanjit S. Aulakh John W. Daly Irwin J. Kopin David Pickar Richard P. Ebstein John F. Tallman | Staff Physician Chief Visiting Fellow Chief Chief Section Chief Visiting Fellow Section Chief | CN NIMH CN NIMH CN NIMH LBC NIADDK LCS NIMH NB NIMH LBC NIADDK NB NIMH |
| PI: Robert M. Cohen OTHER: Dennis L. Murphy Charanjit S. Aulakh John W. Daly Irwin J. Kopin David Pickar Richard P. Ebstein John F. Tallman | Staff Physician Chief Visiting Fellow Chief Chief Section Chief Visiting Fellow Section Chief | CN NIMH CN NIMH CN NIMH LBC NIADDK LCS NIMH NB NIMH LBC NIADDK NB NIMH | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">Iain C. Campbell, Maudsley Hospital, London, England</p> | | | | | |
| LAB/BRANCH <p style="text-align: center;">Clinical Neuropharmacology Branch</p> | | | | | |
| SECTION | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; border-right: 1px solid black; padding-right: 5px;"> TOTAL MANYEARS: <p style="text-align: center;">3.0</p> </td> <td style="width: 33%; border-right: 1px solid black; padding-right: 5px;"> PROFESSIONAL: <p style="text-align: center;">1.5</p> </td> <td style="width: 33%; padding-left: 5px;"> OTHER: <p style="text-align: center;">1.5</p> </td> </tr> </table> | | | TOTAL MANYEARS: <p style="text-align: center;">3.0</p> | PROFESSIONAL: <p style="text-align: center;">1.5</p> | OTHER: <p style="text-align: center;">1.5</p> |
| TOTAL MANYEARS: <p style="text-align: center;">3.0</p> | PROFESSIONAL: <p style="text-align: center;">1.5</p> | OTHER: <p style="text-align: center;">1.5</p> | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input checked="" type="checkbox"/> (c) NEITHER </div> </div> <div style="margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> Evidence has been obtained that <u>clorgyline</u>, a selective type A <u>monoamine oxidase inhibitor</u> (MAOI) with <u>antidepressant</u> properties, leads to decreases in the number of <u>β-adrenergic receptors</u> in rat brain, while pargyline, a selective type B MAOI, an ineffective antidepressant in our human studies, does not. A sequential study of these effects suggests that the clorgyline-induced <u>β-adrenergic receptor</u> changes are secondary to alterations in <u>presynaptic mechanisms</u>. These findings based on radioligand binding studies have now been supported by physiological studies of locomotor activity in response to the specific <u>α_2-adrenergic agonist clonidine</u>, and of <u>in vitro catecholamine release</u> in microsacs prepared from saline-treated and clorgyline-treated animals. The presynaptic changes are observed by three weeks of clorgyline treatment, whereas the post-synaptic physiological changes in <u>norepinephrine stimulated cAMP</u> occur some two weeks later. The MAOI side effects involving sleep and the cardiovascular system also appear to be adaptive in nature and selective for the type A MAOI. Pithed rats show enhanced <u>blood pressure</u> changes to sympathetic stimulation following clorgyline, but not <u>deprenyl</u> treatment. </p> | | | | | |

Project Description:

Objectives: Various antidepressant drugs enhance catecholamine functional activity rapidly in animal models; however, antidepressant effects and some side effects in man are not observable until 10-14 days or longer. This implies that central nervous system adaptive changes are likely to be important in the molecular effects and side effects of these drugs. We have engaged in a series of studies to define this process in rats with the expectation that these studies will further our understanding of the etiology and treatment of affective disorders.

Methods Employed:

Monoamine oxidase activity is determined with [^3H]-serotonin, [^{14}C]-phenylethylamine and [^{14}C]-tyramine as substrates with subsequent separation of labelled products by ion change chromatography. Receptors from crude brain homogenates are measured by standard radioactive ligand assays. [^3H]-dihydroalprenolol, [^3H]-WB4101, [^3H]-clonidine and [^3H]-yohimbine are the specific ligands used for the measurement of β -, α_1 -, and α_2 -receptors respectively. Cyclic AMP formation was measured by the adenine prelabeling brain slice technique. Norepinephrine release experiments were performed using microsacs prepared from rat cortex and layered onto sephadex G 10 columns.

For the cardiovascular studies a pithed rat preparation was utilized with both vagus nerves cut at the neck. This procedure destroys the entire central nervous system but leaves intact the emerging nerve terminals. Plasma norepinephrine and epinephrine were assayed by a radioenzymatic thin layer chromatographic procedure using catechol-o-methyl transferase and [^3H]-S-adenosylmethionine.

Locomotor activity was measured with Animex activity meters.

Major Findings:

The effects of chronic administration of the non-selective monoamine oxidase inhibitor, phenelzine, (15 mg/kg), and two selective inhibitors, clorgyline (1 mg/kg) and pargyline (1 mg/kg) on adrenergic receptor binding and MAO activity were studied in the rat brain. Chronic, but not acute administration of both phenelzine and the MAO-A inhibitor clorgyline resulted in significant decreases in cortical β -adrenoreceptor binding of 47% and 24% respectively. In contrast pargyline, a type B inhibitor which only partially inhibited type A, caused only a small (7%) nonsignificant change in rat cortical β -receptor binding. In a more detailed study of clorgyline, a change in α_2 -adrenergic cortical receptor ([^3H]-clonidine) binding was observable by three days whereas changes in α -([^3H]-WB4101) and β -([^3H]-dihydroalprenolol) cortical receptor binding was not present until Day 10. The rapidity and magnitude of the α_2 changes (62% in cortex and 60% in brainstem) suggested that these changes may contribute to post-synaptic α_1 - and β -receptor changes. To provide further support for the radioligand binding studies, behavioral and physiological correlates of auto-receptor function were examined. Treatment for 21 days, but not 3 days, with

clorgyline caused significant escape from clonidine's normal suppressant effect on locomotion in the rat. For example, 150 $\mu\text{g/kg}$ of clonidine caused a 77% reduction of activity in controls but only a 38% decrease in the clorgyline-treated group. These findings suggested an alteration in the presynaptic inhibitory noradrenergic system.

Microsacs produced from 21-day but not 3-day clorgyline-treated animals were observed to have an increase in norepinephrine release in response to electrolyte stimulation. This increase was accompanied by a change in the responsivity of release to clonidine suppression, and may, in part, correspond to what has been previously referred to as "leakage". For example, catecholamine release at 0.05 mM CaCl_2 and 43 mM K^+ was 981 units for control animals and 1636 units for clorgyline-treated animals. 0.1 μM clonidine reduced release in controls by 46% but only by 6% in clorgyline-treated animals.

Although β -receptor changes were observed in these animals by 21 days, no significant decrease in the cAMP system response to norepinephrine stimulation was observed before 35 days of clorgyline treatment. These results provide direct physiological support for changes in norepinephrine release mechanisms and an effect on the autoreceptor specifically, preceding post-synaptic adaptive changes in the instance of one antidepressant, clorgyline.

Cardiovascular and Sleep Studies: Chronic (21 days) selective MAO-A but not MAO-B inhibition led to increased blood pressure responses to sympathetic stimulation and intravenous tyramine, and to elevated unstimulated heart rates, in pithed rats. No significant changes were observed in plasma catecholamine responses to sympathetic stimulation, nor in β -adrenoreceptor numbers in heart ventricles. This suggests that the hypotensive effects of MAOI's result from CNS rather than peripheral nervous system alternations.

In collaboration with Dr. Mendelson of the Unit on Sleep Studies the effects of clorgyline on the sleep of the rat were examined after subacute and lifetime administration. Subacute administration (2 mg/kg/24 hr for 60 hr) resulted in a 92% inhibition of MAO-A in brain and a significant reduction in REM sleep time. In comparison, fetal rats which were exposed to 1 mg/kg of clorgyline daily were administered 1 mg/kg of clorgyline for six weeks postnatally. Although the animals' MAO-A activity was 1% of their control counterparts no significant EEG sleep stage changes were observed.

Significance to Biomedical Research and the Program of the Institute:

We had previously proposed that an overly stringent negative feedback system could impair the capacity of the catecholamine pathways to convey information adequately during depression. The present data supports the role of some antidepressants in potentially resetting this mechanism as an important component of the molecular process of antidepressant efficacy. Human studies prompted by these types of observations have already revealed changes in some functional measures of α_2 sensitivity following antidepressant treatment in depressed subjects. These studies of adaptive changes are also likely to have relevance for the understanding of the side effects of monoamine oxidase inhibiting antidepressants. As in the instance of antidepressant efficacy, behavioral

(hypomania and mania), sleep and cardiovascular side effects are delayed in onset in man. The rat studies support the hypothesis that tyramine pressor responses (the cheese effect) may be more closely linked to intraneuronal levels of norepinephrine and therefore to MAO-A inhibition than to a reduced enzymatic capacity to deaminate exogenous tyramine. As a result of the observations of increased blood pressor response to sympathetic stimulation in the clorgyline-treated animals, it seems likely that in man the delayed hypotensive effects are likely to be the result of MAO-A inhibition and of subsequent CNS adaptation and not the result of peripheral ganglionic blockade or the formation of false neurotransmitters as has been previously proposed. The findings of adaptation in the REM response may provide a model for further study of REM breakthrough which occurs in some patients receiving MAOI's over long periods of time. An additional finding of differences in the levels of MAO activity remaining in different organs following MAOI treatment in the rat as well as differences in receptor adaptation in different organs observed suggests care in the interpretation of clinical studies that utilize peripheral measures of receptors and MAO inhibition to reflect CNS events.

Proposed Course:

During the next year, we will be attempting to extend our study of antidepressant effects to self-stimulation behavior and to some tricyclic drugs. We will also be looking at adaptive changes in stress models and the effect of specific drugs, e.g., alcohol on these changes. Some properties of antidepressant withdrawal will also be examined. Finally, a review of receptor changes in animal models of state changes will be completed along with a specific hypothesis of our relevance of such models for understanding psychiatric illness.

Publications:

Cohen, R.M., Campbell, I.C., Dauphin, M., Tallman, J.F., and Murphy, D.L.: Changes in α - and β -receptor densities in rat brain as a result of treatment with monoamine oxidase inhibiting antidepressants. Neuropharmacology 21: 293-298, 1982.

Mendelson, W.B., Cohen, R.M., Campbell, I.C., Murphy, D.L., Gillin, J. C. and Wyatt, R.J.: Lifetime monoamine oxidase inhibition and sleep. Pharmacol. Biochem. 16: 429-431, 1982.

Cohen, R.M., Campbell, I.C., Yamaguchi, I., Pickar, D., Kopin, I.J., and Murphy, D.L.: Cardiovascular changes in response to selective monoamine oxidase inhibition in the rat. Eur. J. Pharmacol., in press.

Cohen, R.M., Aulakh, C.S., Campbell, I.C., and Murphy, D.L.: Functional subsensitivity of alpha 2 adrenoreceptors accompanies reductions in yohimbine binding after clorgyline treatment. Eur. J. Pharmacol., in press.

Cohen, R.M., Ebstein, R.P., Daly, J.W., and Murphy, D.L.: Chronic effects of a monoamine oxidase-inhibiting antidepressant: Decreases in functional alpha-adrenergic autoreceptors precede the decrease in norepinephrine stimulated cyclic AMP systems in rat brain. J. Neurosci., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00334-04 CN | | | | | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Studies of the pressor, metabolic and behavioral responses to intravenous tyramine.</p> | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">David Pickar</td> <td style="width: 33%;">Staff Psychiatrist</td> <td style="width: 33%;">BP NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Dennis L. Murphy</td> <td>Chief</td> <td>CNP NIMH</td> </tr> <tr> <td></td> <td>Robert M. Cohen</td> <td>Staff Psychiatrist</td> <td>CNB NIMH</td> </tr> <tr> <td></td> <td>D. C. Jimerson</td> <td>Staff Psychiatrist</td> <td>LCS NIMH</td> </tr> </table> | | | PI: | David Pickar | Staff Psychiatrist | BP NIMH | OTHER: | Dennis L. Murphy | Chief | CNP NIMH | | Robert M. Cohen | Staff Psychiatrist | CNB NIMH | | D. C. Jimerson | Staff Psychiatrist | LCS NIMH |
| PI: | David Pickar | Staff Psychiatrist | BP NIMH | | | | | | | | | | | | | | | |
| OTHER: | Dennis L. Murphy | Chief | CNP NIMH | | | | | | | | | | | | | | | |
| | Robert M. Cohen | Staff Psychiatrist | CNB NIMH | | | | | | | | | | | | | | | |
| | D. C. Jimerson | Staff Psychiatrist | LCS NIMH | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">C.R. Lake, United Services, Univ. of the Health Sciences Medical School, Bethesda, Maryland</p> | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Clinical Neuropharmacology Branch</p> | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">0</div> | PROFESSIONAL: <div style="text-align: center;">0</div> | OTHER: <div style="text-align: center;">0</div> | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | |
| <p>This project has been completed and discontinued. Its principal findings from the three publications listed below were summarized in last year's report.</p> | | | | | | | | | | | | | | | | | | |

Publications:

Pickar, D., Lake, C.R., Cohen, R.M., Jimerson, D.C., and Murphy, D.L.: Alterations in noradrenergic function during clorgyline treatment. Commun. Psychopharmacol. 4: 379-386, 1980.

Pickar, D., Cohen, R.M., Jimerson, D.C., and Murphy, D.L.: Tyramine infusions and selective MAO inhibitor treatment. I. Changes in pressor sensitivity. Psychopharmacology 74: 4-7, 1981.

Pickar, D., Cohen, R.M., Jimerson, D.C., Lake, R.L., and Murphy, D.L.: Tyramine infusions and selective MAO inhibitor treatment. II. Interrelationships among pressor sensitivity changes, platelet MAO inhibition and plasma MHPG reduction. Psychopharmacology 74: 8-12, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00335-04 CN |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Smooth Pursuit Eye Tracking Impairment and Its Relation to Psychopathology and CNS Disorders | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Larry Siever | Staff Physician CNB NIMH |
| OTHERS: | Dennis L. Murphy Monte S. Buchsbaum Theodore P. Zahn Douglas Reingold Peter Bernad Susomo Sato Daniel P. Van Kammen Thomas R. Insel John I. Nurnberger Jean A. Hamilton | Chief Section Chief Research Psychologist Biologist Visiting Scientist Section Chief Staff Physician Staff Physician Staff Physician Staff Physician |
| | | CNB NIMH BP NIMH LPP NIMH CB NEI CN NINCDS CN NINCDS NIMH CNB NIMH BPB NIMH CNB NIMH |
| COOPERATING UNITS (if any) Philip Holzman, Harvard University; Leslie Brody, Children's Hospital, Los Angeles, CA; Robert Coursey, University of Maryland | | |
| LAB/BRANCH Clinical Neuropharmacology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.4 | PROFESSIONAL: 0.8 | OTHER: 0.6 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> This project aims to characterize psychological and psychobiological attributes that are associated with alterations in <u>smooth pursuit eye movements</u>. The smooth pursuit system regulates the eye movements in following a target and can be measured by an <u>electro-oculograph</u> recording of an individual watching a swinging pendulum. By using the <u>high-risk strategy</u> of screening a large group of college student <u>normal volunteers</u>, individuals with various alterations in smooth pursuit patterns have been identified. Individuals with impaired tracking have been compared with those with more accurate tracking using a variety of psychological and biological tests to explore the relationship of these patterns to possible <u>psychopathology</u>, as well as other psychological and biochemical variables. Initial results indicate that schizotypal psychopathology marked by social isolation, suspiciousness, constricted affect, and poor rapport was significantly more likely to be found in low accuracy trackers than high accuracy trackers. </p> | | |

Project Description:

Objectives: This project is designed to study individual variations in smooth pursuit eye movements and their relationship to cognitive controls, personality tendencies, other psychophysiological characteristics, and psychopathology. In order to understand such relationships, we are employing the "biological high risk strategy," i.e., identifying individuals from a population of normal volunteers with extreme values of a biologic variable implicated in the etiology of psychiatric disorders and comparing them psychologically with volunteers with normal values for that variable. This strategy has been employed successfully in studies of monoamine oxidase levels in man. College student normal volunteers have been screened to determine the quality of their smooth pursuit tracking. Subgroups of these students showing definite alterations in their smooth pursuit patterns have been compared to those with extremely accurate tracking on a variety of psychological, biochemical, and psychophysiological tests.

Methods Employed:

Volunteers from a local college, after giving informed consent, participated in a screening session testing their smooth pursuit eye movements. They were instructed to follow a moving pendulum, which made a target excursion of 20 degrees of visual angle and oscillated at 0.4 Hz. Their eye movements were measured via surface electrodes by an electro-oculograph (EOG) amplifier and recorder and both eye position and velocity were recorded on paper.

These records were scored for velocity arrests, i.e., points at which the eye stops moving while the target continues, and the pattern of deviation of the eye movements. For example, there are individuals with (1) a high velocity arrest score and "spikey" pattern of eye movements, as well as (2) individuals with difficulty initiating eye tracking, so that saccades replace the smooth pursuit movements, i.e., "saccadic substitution." A group of individuals from each of these two groups was compared with a matched control group exhibiting exceptionally good tracking patterns using a variety of psychological and biological tests as well as a clinical interview and family history. Low accuracy trackers were retested under laboratory conditions by both EOG and an infrared eye tracking device. The infrared detected eye signal was recorded on FM tape and analyzed by a frequency analyzer, courtesy of Dr. Philip Holzman, to measure the signal-to-noise ratio (an index of accuracy) of the eye movements.

Major Findings:

Two hundred eighty volunteers have been screened and the accuracy of their smooth-pursuit eye movements assessed. Subgroups of individuals with low accuracy tracking, and high accuracy tracking, have been identified and have undergone extensive psychologic testing including Wexler Adult Intelligence Scale, Rorschach, Minnesota Multiphasic Inventory, and a variety of cognitive and perceptual tests. A group of 50 have come to the NIH Clinical Center for psychophysiological studies with Dr. Buchsbaum (Continuous Performance Task), Dr. Zahn (reaction time), neurological exam and EEG with Dr. Brody or Dr. Bernad, and extensive psychiatric interviews including the Schedule for Affective Disorder and Schizophrenia (SADS) and family history with Dr. Siever.

Low accuracy trackers showed evidence of more psychopathology than high accuracy trackers in the psychiatric interview conducted by staff blind to their eye-tracking status. Nine of 11 individuals satisfying DSM-III criteria for Schizotypal Personality Disorder were in the low accuracy tracking group, as were all eight individuals with a history of hypomania by Research Diagnostic Criteria (RDC). Schizophrenia-related characteristics were found in 21/31 of the low accuracy trackers. Further correlations with clinical ratings indicated significant associations between degree of accuracy of tracking and characteristics of flattened affect, decreased rapport, referential ideas, suspiciousness, brief delusions, social isolation, dependency, and preference for being alone.

Preliminary results indicated that low accuracy tracking also correlated with the number of neurological soft signs, abnormalities in EEG, delayed visual evoked potentials, and left-handedness, although most of these results were within clinically normal limits. Correlations also emerged between tracking inaccuracy and the Chapman anhedonia scale, embedded figures test, and other psychological test data.

Results from this study are still being analyzed, but suggest that low accuracy tracking is associated with schizophrenia-related and mania-related traits. In these subjects with schizophrenia-related traits, the results reflected the profile of an individual with subtle neurologic dysfunction, poor perceptual boundary differentiation, decreased affect, and social isolation with avoidance of interpersonal intimacy. We have now retested the majority of low accuracy trackers and find that 11 out of the 13 subjects who show low accuracy tracking under laboratory conditions manifest some schizotypal characteristics.

We also have studied the correlation between inaccuracy of tracking and behavioral response to amphetamine in psychiatric patients, to test the hypothesis that arousal may enhance psychosis-related behaviors in these vulnerable individuals. These results suggest that low accuracy trackers improve their tracking with amphetamine while it deteriorates in individuals with high accuracy tracking.

Finally, we are evaluating correlations between inaccuracy of tracking, psychosis-related characteristics and other biologic variables in various psychiatric patient groups including obsessive-compulsives, affectively disordered patients, and schizophrenics in collaboration with Dr. Nurnberger, Dr. Thomas Insel, Dr. Daniel van Kammen, and others.

We have observed inaccurate tracking in schizophrenics who have been off medication for at least 10 days, supporting previous evidence that this abnormality does not represent a medication effect. We have not observed any significant correlations to date with biologic variables such as cerebrospinal fluid amine metabolite levels or ventricular size as indicated by the CAT scan, or clinical variables such as premorbid history.

In a related substudy conducted by Jean Hamilton, the high and low accuracy eye-tracking groups are being compared on a number of additional personality variables, including brief self-report measures of interest and boredom that have been previously validated in work done at NIMH by Dr. Hamilton, in association

with Monte Buchsbaum's group. Boredom has been a relatively neglected research topic except for Zuckerman's "Boredom Susceptibility" subscale. However, both intrinsic interest and boredom are "affects" that accompany the cognitive information-processing act in attention and the prominence of attention as an explanatory construct in schizophrenia and hyperactivity suggests taking a closer look at the role of boredom in psychopathology. Since poor eye-trackers are hypothesized to have a deficit in "automatic" or involuntary attentional control mechanisms, we predicted that this group would report more boredom and less capacity to focus their attention in a smoothly regulated way that would ensure intrinsic enjoyment. The data evaluating this hypothesis are concurrently being examined.

Significance to Biomedical Research and the Program of the Institute:

The smooth pursuit system has been a focus of interest in psychiatry since it has been demonstrated that it is significantly impaired in psychotic individuals, particularly schizophrenics, as well as some patients with severe affective disorders. This is hypothesized to represent a deficit in involuntary attention or cognitive centering. "Good" and "poor" tracking patterns have been shown to be under genetic control and thus to represent one of the few genetically biologic variables consistently associated with schizophrenia and other psychoses. By using the high-risk strategy of screening a college population for alterations in this system, the relationship of this variable to qualities of attention and personality tendencies in a non-psychiatrically defined population might be clarified without the confounding variables of the length of illness, medication or hospitalization. This may facilitate delineating a dimension of psychological function and psychopathology closely tied to involuntary attention.

Proposed Course:

We will continue to study the 50 selected volunteers until as many as possible have undergone psychophysiological and neurologic testing, a psychiatric interview, and repeat eye-tracking evaluation, as well as other procedures. We are also continuing to study schizophrenic, obsessive-compulsive and affectively ill patients. The major current effort is directed toward pulling together and writing up the first results from the high-risk study group, emphasizing the structured interview data.

Publications:

Siever, L.J., Coursey, R.D., Alterman, I.S., Buchsbaum, M.S., and Murphy, D.L.: Psychological and physiological correlates of variations in smooth pursuit eye movements. In Usdin, R. and Hanin, I. (Eds.): Biological Markers in Psychiatry and Neurology. New York, Pergamon Press, 1982, pp. 359-370.

Siever, L.J., Haier, R.J., Coursey, R., Murphy, D.L., Holzman, P.H., Brody, L., Weingartner, H.L., Sostek, A.J., and Buchsbaum, M.S.: Smooth pursuit eye movements in non-psychiatric populations: Relationship to other "markers" for schizophrenia and psychological correlates. Arch. Gen. Psychiatry, in press.

Hamilton, J.A.: Attention, personaiity, and the self-regulation of mood:
Absorbing interest and boredom. In Maher, B.A. (Ed.): Progress in Experimental
Personality Research, New York, Academic Press, 1981, vol. 10, pp. 281-314.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00336-03 CN | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | Monte S. Buchsbaum | Section Chief | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Carol F. Hoover | Social Worker | CN | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Judith L. Rapoport | Unit Chief | BP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Jean Hamilton | Staff Physician | CN | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | Theodore Zahn | Staff Psychologist | LPP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| <p>Obsessive-compulsive disorder is an uncommon syndrome about which little is known. The purpose of this project is to collect basic data about the phenomenology, the psychobiology, and the treatment of this disorder. Initial results with several biologic variables which have previously been described as abnormal in patients with depression suggest that obsessive-compulsive disorder patients share some of the psychobiologic features of affective illness. These variables include dexamethasone suppression, sleep physiology, and average evoked response. Furthermore, preliminary data suggests that patients with obsessive-compulsive disorder may respond to the antidepressant, clomipramine.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: Over the past two years we have developed a comprehensive research program in both psychological and biological aspects of obsessive-compulsive disorder. There were three short range objectives of this program for the past year. First, we needed to develop a taxonomy of this disorder. Is obsessive-compulsive disorder a homogeneous entity or should we speak of obsessional disorders with heterogeneous responses to treatment? Second, we were interested in exploring some of the psychobiologic variables previously found abnormal in either affective disorder or schizophrenia to find out if obsessional patients would show these abnormalities and, if so, whether they would resemble affective disorder patients more than schizophrenics. Finally, we set out to add to the scant knowledge on the pharmacologic treatment of this disorder. Would these patients benefit from antidepressant treatment? Would a tricyclic antidepressant be more effective than a monoamine oxidase inhibitor, and would either be superior to placebo?

Methods Employed:

Obsessive-compulsive subjects were studied in both inpatient and outpatient settings. Patients who applied for the protocol were carefully screened with an extensive psychological and medical battery. Those accepted had obsessive or compulsive symptoms for at least one year independent of another psychiatric diagnosis. An initial evaluation included cognitive, projective, psycholinguistic and psychophysiology (EEG, GSR, AER) testing. Biologic measures which have proved useful in studies of affective or schizophrenic disorders were focused on here: sleep physiology, average evoked response, urinary and cerebrospinal fluid monoamine metabolites, a variety of platelet and RBC enzymes, and the dexamethasone test were all studied. Cerebral CT scans were done to investigate the possibility of gross neuropathology in this disorder. A major aspect of the study was the comparative double-blind drug trials. This trial followed a randomized cross-over design with assessment by both self and observer ratings on a weekly basis. Specific biological and psychological measures were repeated during the various stages of the drug trial for correlation with clinical state.

An additional study completed within the last year examined behavioral and neuroendocrine responses to amphetamines as predictors of clinical response to clomipramine or clorgyline. The theory, borrowed from analogous studies in depressed patients, had been that those patients developing activation or euphoriant response to a single dose of d-amphetamine would be more likely to respond therapeutically to one of the drugs in the double-blind crossover study.

Major Findings:

A total of 27 patients with obsessive-compulsive disorder have been studied in one or more parts of this project during the past two years. As the disorder is uncommon, much of the first year was devoted to recruitment. The past year has been devoted to data collection and analysis. We are just beginning a second generation of studies based on these results.

While our original question about taxonomy has not been completely resolved, we have addressed several aspects of this problem. Defining subgroups on the basis of ritualizers (i.e. compulsives) versus ruminators (i.e. pure obsessionals) as has been done elsewhere, has not proved valid on the basis of historical data, psychophysiologic measures, or treatment responses. Similarly, separating ritualizing patients on the basis of types of rituals (i.e., cleaners versus checkers) has not led to predictable differences in psychophysiologic or treatment response measures. Our tentative scheme to identify subgroups is the recognition of differences in highest level of premorbid functioning. One subgroup of obsessionals, whom we have called narcissistic, has a history of childhood onset, absence of affective-like biologic abnormalities, and requires major psychosocial intervention for treatment, including family therapy. A second subgroup, whom we have called affective, is more likely to show onset during adulthood, to manifest biologic indices of affective illness, and to ultimately have a somewhat better prognosis.

Psychobiologic findings in obsessionals have consistently resembled abnormalities in patients with affective illness. Dexamethasone suppression tests were abnormal with 6 of 16 subjects (37.5%) showing escape from suppression after 1 mg of dexamethasone. Sleep electroencephalography in 14 obsessionals revealed a pattern of shallow, fragmented sleep with short latency to the first rapid eye movement (REM) period. Comparing these polygraphic records to records from a group of age-matched normals revealed marked differences from the normals on measures of sleep continuity, sleep architecture, and REM sleep. An identical comparison with age-matched depressives found that obsessionals could be distinguished from the depressives on only a few subtle measures of REM sleep (REM density and REM efficiency). These sleep abnormalities were not restricted to obsessionals with secondary depression. On measures of averaged evoked response, the obsessionals once again resembled patients with affective illness. Unlike schizophrenics, they showed an increased amplitude for visual evoked responses. In line with certain affective subgroups, they showed a tendency to not increase the amplitude of these responses with increasing stimulus intensity. While these data from neuroendocrine tests, sleep physiology, and evoked potentials might all be used to question the diagnostic specificity of either the measures or the syndrome, we feel that a more heuristic interpretation is that obsessional patients share some biologic substrate with affective illness patients. Data from treatment response further support this formulation.

Thus far, 13 patients have completed a randomized, placebo controlled, double-blind crossover study of clomipramine and clorgyline. Preliminary results suggest consistent improvement with clomipramine, even in those patients without depressive symptoms. Clorgyline and placebo appear to be less effective.

While data are not yet complete on whether behavioral response differences to d-amphetamine will predict which patients improve, a chance finding in the amphetamine study suggests a powerful though transient improvement in obsessional symptoms, possibly related to the activating effect of the drug.

Significance to Biomedical Research and the Program of the Institute:

These studies of obsessive-compulsive disorder represent the most comprehensive investigations yet done in this country on this uncommon illness. The biological evidence added to clinical data points to the similarities between this syndrome and disorders of affect. That these patients respond to a tricyclic antidepressant further supports this link between the two disorders and raises new optimism for an illness which has heretofore been considered refractory to treatment.

Proposed Course:

A second generation of studies will focus on the mechanism of improvement with clomipramine. Data collected in the initial crossover study will be analyzed to tell us more about psychophysiologic and psychobiological changes on each of the two drugs. As clomipramine is a complex pharmacologic agent with effects on several monoamine systems, we have just begun a study comparing two more selective antidepressants: desmethylinipramine and zimelidine. We hope that a differential response to these two compounds along with changes in cerebrospinal fluid monoamine metabolites will tell us more about the biochemical changes necessary for the anti-obsessional effect. Further study using the challenge paradigm with amphetamine and also with naloxone and fenfluramine should supplement the treatment study to tell us more about the pharmacology of obsessions.

Publications:

Insel, T. and Murphy, D.L.: The psychopharmacologic treatment of obsessive-compulsive disorder: A review. J. Clin. Psychopharmacol. 1(4): 207-213, 1981.

Insel, T., Kalin, N., Guttmacher, L.B., Cohen, R.M., and Murphy, D.L.: The dexamethasone suppression test in patients with obsessive-compulsive disorder. Psychiatry Res. 6: 153-160, 1982.

Insel, T.R.: Obsessive-compulsive disorder: Five clinical questions and a suggested approach. Comp. Psychiatry 23: 241-251, 1982.

Insel, T.R., Gillin, J.C., Moore, A., Loewenstein, R., Mendelson, W., and Murphy, D.L.: The sleep of obsessive-compulsive disorder patients. Arch. Gen. Psychiatry, in press.

Insel, T.R., Alterman, I., and Murphy, D.L.: Anti-obsessional and antidepressant responses to clomipramine. Psychopharmacol. Bull., in press.

Insel, T.R., Roy, B., Cohen, R.M., and Murphy, D.L.: Development of the serotonin syndrome in man. Am. J. Psychiatry, in press.

Linnoila, M., Insel, T.R., Kilts, C., Potter, W.Z., and Murphy, D.L.: Plasma steady-state concentrations of hydroxylated metabolites of clomipramine. Clin. Pharmacol. Ther., in press.

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| | Lawrence Tamarkin | Staff Fellow | IRP | NICHD | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | Steven Paul | Chief | NB | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Ned Kalin, Wm. T. McKinney and Gary W. Kraemer, Wisconsin Primate Lab; Craig Risch, Univ. of CA, San Diego; Margery Beinfeld, St. Louis Univ., St. Louis, MO; Philip Taylor, Center for Reprod. Biology, Edinburgh, Scotland | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Clinical Neuropharmacology Branch</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) We have continued our studies of <u>cerebrospinal fluid</u> (CSF) levels of various hormones, peptides, and monoamine metabolites in <u>rhesus monkeys</u> : (1) Studies with prolactin have demonstrated a significant diurnal rhythm in CSF as well as a blood-CSF gradient; a ventricular-lumbar gradient within the CSF is not present under baseline conditions but develops following treatment with thyrotropin stimulating hormone, which stimulates prolactin release; (2) an investigation of CSF <u>serotonin</u> and <u>melatonin</u> concentrations sampled over a 24-hour period revealed a marked diurnal rhythm in serotonin which was nearly identical in timing to the melatonin rhythm; (3) studies of CSF <u>cholecystokinin</u> have shown no response to intravenous amphetamine administration. These investigations are beginning to clarify the potential effects of some neuro-endocrine substances on the brain. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: The discovery that a multitude of peripheral peptide hormones are present in high concentrations in the brain has led to an entire field of inquiry into the role of hormones as modulators of the classical monoamine neurotransmitters. This project has focused on the measurement of these peptides in cerebrospinal fluid in an attempt to (a) define the relationship between plasma and CSF peptide levels; (b) sort out their concentrations at different levels of CSF (i.e., lateral ventricular versus lumbar); (c) monitor diurnal changes; and (d) assess the effects of drugs which are known to affect monoamines.

Methods Employed:

Cerebrospinal fluid from non-human primates is collected by means of indwelling lumbar or lateral ventricular cannulae for continuous flow into a refrigerated fraction collector. Monkey plasma is obtained either by use of indwelling venous catheters or by femoral venipuncture following ketamine induced anesthesia. The following hormones are measured by radioimmunoassay: cortisol, prolactin, growth hormone, β -endorphin, melatonin (in collaboration with Larry Tamarkin), vasopressin (in collaboration with Robert Zerbe), and cholecystokinin (in collaboration with Margery Beinfeld). Monoamines and monoamine metabolites are measured by reverse phase high performance liquid chromatography with electrochemical detection (in collaboration with Markku Linnoila).

Major Findings:

Studies of prolactin, coordinated by Dr. Kalin, revealed a circadian prolactin rhythm in CSF similar to the well-described rhythm in plasma. Further studies employing intravenous administration of radioactively-labeled prolactin revealed a delayed peak in CSF at 1-3 hours following injection. Pharmacologically stimulated prolactin release, using thyrotropin releasing hormone (TRH) revealed a similar latency for CSF compared to plasma response. No ventricular-lumbar gradient for prolactin within CSF was evident in the baseline state.

A pronounced diurnal variation has been demonstrated for the first time in rhesus monkey lumbar and ventricular cerebrospinal fluid serotonin concentrations. A gas chromatographic-mass spectrometric method developed last year by Philip Taylor and Sanford Markey of the LCS provided a sensitive assay system for these studies. CSF melatonin measured in the same sample by radioimmunoassay followed a temporally identical pattern, with concentrations rising at the beginning of dark onset, peaking at 2400 hours and falling to baseline at the onset of lighting. Ventricular serotonin concentrations were 3.5-fold higher than lumbar concentrations. Nighttime serotonin concentrations were elevated 0- to 50-fold over daytime levels.

Although five of the seven monkeys studied showed a clear diurnal serotonin rhythm, the two who failed to show a nighttime increase in serotonin also lacked a rhythm in melatonin. Individual monkeys studied repeatedly showed highly consistent day-to-day diurnal amplitude variations.

Other studies in this project are currently in the data collection stage, although several preliminary findings are of interest.

Studies with cholecystokinin (CCK) have shown no evidence of a circadian rhythm. Amphetamine administration, known to increase dopamine release in brain, did not lead to a release of CCK. Studies with CCK changes after feeding are in progress.

Studies with vasopressin have similarly not found a circadian rhythm. Early data suggest a reverse gradient (lumbar greater than ventricular levels) and a robust response of CSF vasopressin to apomorphine administration.

Studies of models of anxiety with lactate and with benzodiazepine antagonists (in collaboration with Steve Paul, NB) are just now looking at plasma and CSF changes in cortisol and monoamines as physiologic responses to stress.

Significance to Biomedical Research and the Program of the Institute:

These studies in subhuman primates provide a valuable approach to the interaction of CSF peptides and monoamines over an extended time period.

Proposed Course:

We hope to complete the studies outlined above, and, in particular extend the investigations of the relationships between the high concentrations of serotonin in the CSF and melatonin and other neuropeptides. In the future we hope to improve our collection system so that the animals will be freely moving rather than chair restrained to permit more behavioral measures to supplement the endocrine data.

Publications:

Kalin, N.H., Insel, T.R., Cohen, R.M., Risch, S.C., and Murphy, D.L.: Diurnal variation in cerebrospinal fluid prolactin concentration of the rhesus monkey. J. Clin. Endocrinol. Metab. 52: 857-858, 1981.

Risch, S.C., Kalin, N.H., Cohen, R.M., Weker, J.L., Insel, T.R., Cohen, M.L., and Murphy, D.L.: Muscarinic cholinergic influences on ACTH and β -endorphin release mechanisms in human subjects. Peptides 2: 95-97, 1981.

Kalin, N.H., Burns, S., Risch, S.C., Cosgrove, S.A., Warden, D.A., and Murphy, D.L.: The relationship between blood and cerebrospinal fluid prolactin in non-human primates. Life Sciences, in press.

Taylor, P.L., Garrick, N.A., Burns, R.S., Tamarkin, L., Murphy, D.L., and Markey, S.P.: Diurnal rhythms of serotonin in monkey cerebrospinal fluid. Life Sciences, in press.

Risch, S.C., Janowsky, D.S., Siever, L.J., Judd, L.L., Rausch, J.L., Huey, L.Y., Beckman, K.A., Cohen, R.M., and Murphy, D.L.: Correlated cholinomimetic-stimulated beta-endorphin and prolactin release in humans. Peptides, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00338-02 CN | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Families of Origin in Obsessive-Compulsive Illness | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: Carol F. Hoover</td> <td style="width: 33%;">Social Worker</td> <td style="width: 33%;">CN NIMH</td> </tr> <tr> <td>PI: Thomas R. Insel</td> <td>Staff Physician</td> <td>CN NIMH</td> </tr> </table> | | | PI: Carol F. Hoover | Social Worker | CN NIMH | PI: Thomas R. Insel | Staff Physician | CN NIMH |
| PI: Carol F. Hoover | Social Worker | CN NIMH | | | | | | |
| PI: Thomas R. Insel | Staff Physician | CN NIMH | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | |
| LAB/BRANCH Clinical Neuropharmacology Branch | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL MANYEARS: 0.3 | PROFESSIONAL: 0.2 | OTHER: 0.1 | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This study explores the etiological context of developing <u>obsessive-compulsive illness</u> , with emphasis on <u>family relationships</u> and <u>interaction</u> , as well as the implications of obsessive-compulsive symptomatology, traits, or personality among relatives. | | | | | | | | |

Project Description:

Objectives: Few intensive studies have been made of the family setting of this illness, and investigations of symptomatology among relatives have left the matter of possible genetic factors in doubt. In the current study, it is intended to develop a schema which takes into account our observations of family relationships, the form of the patient's illness, and the nature as well as extent of illness among relatives.

Methods Employed:

Individual and conjoint interviews were held with severely obsessive-compulsive patients and members of their families. Information has been collected on 10 patients, 40 parents and siblings, plus 134 other relatives. The Leyton Obsessional Inventory has been administered to patients and their parents.

Major Findings:

Among the 174 relatives of these severely ill patients, there were none who suffered from classical compulsions, although mental illnesses of other types (e.g., affective illness) were not uncommon. Parents in these families rejected the virtually delusional beliefs of the obsessive-compulsive patient, even when bullied into following the most bizarre demands, and did not develop compulsions or rituals of their own. However, the relatives in these families had developed overmeticulous, superclean, perfectionist attitudes which became part of the family culture through the generations. The perfectionist traits of the parents did not cause them discomfort or interfere with their lives, but did prevent early recognition of the patient's increasingly abnormal course as a child and young adult.

Typically in these families at least one parent also projected upon the child unfulfilled marital closeness needs, and the patient's obsessive-compulsive symptoms served as a protective barrier against being overwhelmed by the parents symbiotic expectations. The patient became a person of extraordinary power in the family, virtually enslaving parents by tantrums and excesses, leading to a mutual entrapped isolation of the family from community life.

Since severe obsessive-compulsive illness has sometimes been considered to border on schizophrenia, it may be noted that identifiable schizophrenia was not evident among relatives. Parental attitudes toward the ill offspring were also quite different: in marked contrast to an across-the-board denial of involvement characteristic of the parents of schizophrenics, the parents of obsessive-compulsive offspring were generally consumed with guilt for their part in the patient's developing illness. These parents were especially ashamed of their inability to say no to the patient's increasingly unreasonable demands.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00339-01 CN | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Neuropharmacology of Cognition | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Robert M. Cohen</td> <td style="width: 33%;">Staff Physician</td> <td style="width: 33%;">CNB NIMH</td> </tr> <tr> <td>OTHER: Dennis L. Murphy</td> <td>Chief</td> <td>CNB NIMH</td> </tr> <tr> <td>Herbert Weingartner</td> <td>Unit Chief</td> <td>LPP NIMH</td> </tr> <tr> <td>David Pickar</td> <td>Section Chief</td> <td>NB NIMH</td> </tr> <tr> <td>Thomas Insel</td> <td>Staff Physician</td> <td>CNB NIMH</td> </tr> </table> | | | PI: Robert M. Cohen | Staff Physician | CNB NIMH | OTHER: Dennis L. Murphy | Chief | CNB NIMH | Herbert Weingartner | Unit Chief | LPP NIMH | David Pickar | Section Chief | NB NIMH | Thomas Insel | Staff Physician | CNB NIMH |
| PI: Robert M. Cohen | Staff Physician | CNB NIMH | | | | | | | | | | | | | | | |
| OTHER: Dennis L. Murphy | Chief | CNB NIMH | | | | | | | | | | | | | | | |
| Herbert Weingartner | Unit Chief | LPP NIMH | | | | | | | | | | | | | | | |
| David Pickar | Section Chief | NB NIMH | | | | | | | | | | | | | | | |
| Thomas Insel | Staff Physician | CNB NIMH | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Neuropharmacology Branch | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.6 | PROFESSIONAL: 0.3 | OTHER: 0.3 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Memory problems are a common complaint in the <u>affective disorders</u> . In prior work reported by Dr. Weingartner of the Laboratory of Psychology and Psychopathology, it was established that some of the memory deficits in depression were alleviated by treatment with the catecholamine enhancing drug, amphetamine. In addition, it was observed that <u>memory performance</u> could be improved by providing an information structure for the data that was to be memorized. These results suggested that a deficit in the <u>central motivational state</u> of depressed subjects might account for their performance problems. To further explore this possibility, an indirect method was devised for the measurement of motivational state based on performance on a motor task. <u>Motor performance</u> and <u>cognitive function</u> were then examined in depressed patients and controls. Increasing severity of depression was strongly associated with decrements in performance in both motor and memory tasks. Greatest depression-related impairment was found in those cognitive and motor tasks that required sustained effort. | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: Memory deficits have been associated with a number of neurotransmitter pathways in specific disease states and in drug-induced states in normals. Although memory performance is a somewhat specialized form of behavior, the study of memory performance requires an understanding of the intersection of motivation, drive and attention with what may be the specific physiological events of remembering (the engram). Therefore, the study of cognition in depression and other neuropsychiatric illnesses may help to elucidate the specific mechanisms whereby the physiologic and biochemical substrates of these illnesses influence the central motivational state and its interaction with the specific processes involved in behavior in general, and in particular of memory. It is probably not coincidental that primary neuropsychiatric illnesses (SLE, Alzheimer's and Korsakoff's) are associated with mood disorders. Therefore, understanding the interrelationships between mood and cognition should aid in the treatment of some of the deficits in these disorders. In the future, the use of drugs to manipulate neurotransmitter systems in both patients and normals is a likely strategy to be explored.

Methods Employed:

Behavioral Assessment: Diagnoses of major affective illness in each of the patients is made on the basis of Research Diagnostic Criteria with the aid of the Schedule for Affective Disorders and Schizophrenia. Degree of depression is measured by the nursing staff utilizing the Bunney-Hamburg 15-point ward rating scale, by physicians with the Hamilton rating scale, and by the subjects using the Beck Depression Inventory and the Profile of Mood States.

The memory task consisted of equivalent lists of 40 items each (an item consisted of three different consonants, e.g. MXP). The subject would hear an item and then have to recall the item at an interval of either 0, 3, 6, 9 or 18 seconds later. The intervals were randomly determined. The motor task was designed to attempt to measure sustained effort which would presumably be related in a positive sense to drive and motivation. Each subject was asked to squeeze a dynamometer as hard as he could first with his right and then with his left hand. The subject was then required to sustain a squeeze at one-half this maximum squeeze for as long as he could. Peak effort in kg and time in seconds were recorded.

Major Findings:

Across both normals and depressed subjects, motor and behavioral ratings showed good consistency. Left with right hand peak responses ($r = 0.95$) and left with right sustained times were highly correlated ($r = 0.77$); POMS depression scale with Hamilton ($r = 0.92$) and Beck ($r = 0.97$) depression scales were also highly correlated. All behavioral ratings had significant negative relationships with respect to motor performance. Similarly, memory performance was inversely related to the behavioral ratings. As groups the normals averaged 6.4 words on recall, whereas severely depressed patients recalled 3.9 words. Severely depressed patients had a peak motor response which averaged 20.5 kg which was sustained at one-half that level for 9 seconds, whereas

normals had a peak of 38.5 kg which was sustained at one-half maximum effort for 26.9 seconds. Also, as expected on the basis of the increasing effort required at longer recall intervals, differences in recall between normals and depressed patients increased as the time of recall increased.

Significance to Biomedical Research and the Program of the Institute:

Cognitive changes frequently accompany the mood disturbances that characterize the affective disorders, with laboratory observations of depressed patients showing a positive correlation between the degree of impairment and the intensity of depression. So striking are these changes that they are frequently assigned a central role both in the etiology and treatment of depression. The finding of a close relationship between the decrements in performance of both motor and cognitive function in depression leads one to propose the parsimonious explanation of a single deficit in the area of control of motivational state in depression. In addition the methods developed can be usefully employed in studying other cognitively impaired populations to dissect the issues of motivation and reinforcement from other types of impairments (e.g. in Alzheimer's and Korsakoff's patients). Drug effects on cognition in these patients must be critically analyzed since motivation and effort may underlie cognitive changes which would otherwise appear to be direct effects on the memory processes themselves.

Proposed Course:

The significance of the changes in the motor task will be pursued by studying the responses of subjects on amphetamine as well as by studying patients with the Korsakoff's and Alzheimer's disorders. We will also be examining the effects of catecholamine inhibiting drugs on mood and memory in Alzheimer's patients.

Publications:

Cohen, R.M., Weingartner, H., Smallberg, S.A., Pickar, D., and Murphy, D.L.: Effort and cognition in depression. Arch. Gen. Psychiatry, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00446-13 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Inpatient Clinical Studies of Affective Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: Frederick Goodwin</td> <td style="width: 40%;">Chief</td> <td style="width: 30%;">CP/NIMH</td> </tr> <tr> <td>OTHER: Thomas Wehr</td> <td>Chief, Clinical Research Unit</td> <td>CP/NIMH</td> </tr> <tr> <td>Yolande Davenport</td> <td>Chief, Unit on Family Studies</td> <td>CP/NIMH</td> </tr> <tr> <td>Phillip Gold</td> <td>Chief, Unit on Neuroendocrinology</td> <td>CP/NIMH</td> </tr> <tr> <td>William Potter</td> <td>Assistant to the Branch Chief</td> <td>CP/NIMH</td> </tr> <tr> <td>Rex Cowdry</td> <td>Chief, Outpatient Unit</td> <td>CP/NIMH</td> </tr> <tr> <td>Steven Paul</td> <td>Chief, Unit on Preclin. Pharma.</td> <td>CP/NIMH</td> </tr> <tr> <td>Markku Linnoila</td> <td>Guest Worker</td> <td>CP/NIMH</td> </tr> <tr> <td>Norman Rosenthal</td> <td>Clinical Associate</td> <td>CP/NIMH</td> </tr> <tr> <td>Richard Ross</td> <td>PRAT Fellow</td> <td>CP/NIMH</td> </tr> <tr> <td>Leslie Becker</td> <td>Clinical Associate</td> <td>CP/NIMH</td> </tr> <tr> <td>Richard Hauger</td> <td>PRAT Fellow</td> <td>CP/NIMH</td> </tr> <tr> <td>David Sack</td> <td>Staff Psychiatrist</td> <td>CP/NIMH</td> </tr> </table> | | | PI: Frederick Goodwin | Chief | CP/NIMH | OTHER: Thomas Wehr | Chief, Clinical Research Unit | CP/NIMH | Yolande Davenport | Chief, Unit on Family Studies | CP/NIMH | Phillip Gold | Chief, Unit on Neuroendocrinology | CP/NIMH | William Potter | Assistant to the Branch Chief | CP/NIMH | Rex Cowdry | Chief, Outpatient Unit | CP/NIMH | Steven Paul | Chief, Unit on Preclin. Pharma. | CP/NIMH | Markku Linnoila | Guest Worker | CP/NIMH | Norman Rosenthal | Clinical Associate | CP/NIMH | Richard Ross | PRAT Fellow | CP/NIMH | Leslie Becker | Clinical Associate | CP/NIMH | Richard Hauger | PRAT Fellow | CP/NIMH | David Sack | Staff Psychiatrist | CP/NIMH |
| PI: Frederick Goodwin | Chief | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| OTHER: Thomas Wehr | Chief, Clinical Research Unit | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Yolande Davenport | Chief, Unit on Family Studies | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phillip Gold | Chief, Unit on Neuroendocrinology | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| William Potter | Assistant to the Branch Chief | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Rex Cowdry | Chief, Outpatient Unit | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Steven Paul | Chief, Unit on Preclin. Pharma. | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Markku Linnoila | Guest Worker | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Norman Rosenthal | Clinical Associate | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Richard Ross | PRAT Fellow | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Leslie Becker | Clinical Associate | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Richard Hauger | PRAT Fellow | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| David Sack | Staff Psychiatrist | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Clinical Branch, NEI | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Psychobiology Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 4.3 | PROFESSIONAL: 2.5 | OTHER: 1.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorder, particularly with regard to the complex interrelationships among clinical phenomenology, diagnosis, biological variables, and pharmacologic response. The studies are both cross-sectional and longitudinal; drug trials and double-blind. Integration of the inpatient and outpatient units has advanced, focusing on a brief pretreatment inpatient evaluation of patients with a variety of endogenous and non-endogenous depressive syndromes, followed by outpatient treatment. Meanwhile, longitudinal inpatient studies of endogenous depression, mania, and rapid-cycling bipolar illness continue. Major projects include the study of circadian and seasonal rhythms, neuroendocrine substances and monoamine neurotransmitters and their metabolites, both during untreated affective episodes and following specific treatment. The primary treatment modalities include (1) environmental manipulations, such as sleep deprivation or sleep shifts; (2) tricyclic antidepressants showing specificity for given neurotransmitter systems; and (3) electroconvulsive therapy (ECT). | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

This description attempts to provide a comprehensive view of the inpatient clinical studies of manic-depressive illness. In this group of studies the overall objective is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorders, particularly in relation to specificity of diagnosis, existence of clinically or biologically identifiable subgroups, the nature of predisposing factors, and the interrelationship between pharmacological and psychodynamic factors in clinical improvement.

Traditionally, the focus of our specialized resources has been the longitudinal study of episodes of affective illness, before, during and often following a variety of therapeutic interventions. Initially, studies focused on classical, discrete episodes of mania or endogenous depression; more recently, our inpatient resources have been devoted increasingly to individuals with rapid-cycling bipolar illness. During the past year we continued to expand the "brief inpatient evaluation" program for depression, in which outpatients are admitted to the unit for one week of pre-treatment studies and are then discharged to be treated as outpatients (see Project Z01 MH 018523-03 CP). This approach enables us to evaluate a much broader spectrum of depressive disorders, using the methodologies previously applied to the endogenous depressions, without seriously compromising our ongoing longitudinal studies of entire illness episodes. A major new group of outpatients with seasonally recurring depression has been studied during summer and winter, and before and after experimental treatment with light. Both the total number of patients studied and number of individual projects has increased. The resulting impact on the ward milieu has been well managed.

Central to an effective program of inpatient studies are regular and reliable procedures for gathering data. A variety of variables are monitored continually during a patient's hospital stay. Trained nursing staff members complete twice-daily global ratings, assessing depression, mania, psychosis, anger, and anxiety. Twice a week a modified form of the Brief Psychiatric Rating Scale (BPRS) is scored for each patient. Patients complete self-rating forms twice a day. Specialized scales, such as the Hamilton Depression Scale, the Beck Depression Inventory, or the Symptom Checklist (SCL-90), are used for particular studies. Information regarding families is obtained through ongoing contact between the social workers and the patient's relatives.

Behavioral data are continually collected and quantified. Sleep is recorded at half-hour intervals throughout the night. Motor activity is recorded continuously using solid-state wrist activity monitors (see Project Z01 MH 00450-07 CP), allowing the application of sophisticated mathematical techniques such as spectral analysis to define hitherto unrecognizable patterns in the data. Finally, samples of plasma and urine are collected at regular intervals and stored for subsequent analysis, providing a "bank" of biochemical specimens from various mood states and treatment. We are currently developing an optical scan computer-based system of automated record keeping for ratings, procedures, etc. It is anticipated that this system will greatly facilitate management of research protocols and data analysis, and will be considerably less labor-intensive than the existing data management systems.

In addition, specific cross-sectional studies are scheduled to provide information about the patient's pretreatment biochemical state and the biochemical changes associated with changes in mood and treatment modality. Among these cross-sectional studies are: baseline neuroendocrine values, circadian neuroendocrine patterns, and responses to neuroendocrine challenges (dexamethasone, saline, and TRH infusions) (see Project Z01 MH 00452-06 CP); circadian patterns of activity and temperature (see Project Z01 MH 00450-07 CP); sleep recordings; and cerebrospinal fluid and urinary monoamines and monoamine metabolites (see Project Z01 MH 00447-12 CP).

Since descriptive and diagnostic dimensions are crucial to the meaningful interpretation of psychobiologic data, work on the standardization of these dimensions has continued. Use of the Research Diagnostic Criteria (RDC) (described in detail in the July 1976 - September 1977 report on Project Z01 MH 00446-08 CP) is now routine. Discharge review conferences are used to establish both a final RDC diagnosis and assess the extent to which each patient has "typical endogenous" depressive episodes. In order to facilitate this task a subjective "typicality" scale is assessed and a record of specific endogenous features is made.

After the evaluation phase of hospitalization, a variety of investigational interventions are employed. Environmental manipulations such as sleep shifts and exposure to bright light have played an increasingly prominent role in the overall research program.

A variety of pharmacologic studies continue on the inpatient unit. All drug trials are conducted under double-blind conditions with alternating active and placebo periods in a non-random design. The use of placebo substitution following active drug increases one's level of confidence in the efficacy of a compound in an individual patient. This strategy is important in the evaluation of drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness.

An extension of previous studies of the behavioral and biochemical effects of electroconvulsive therapy (ECT) is under way with increased emphasis on neuroendocrine and circadian parameters.

Results of these interventions are described in greater detail in the individual project reports.

Finally, "normal volunteers" live on the inpatient unit for varying periods of time to participate in specific investigational studies. During the past year, they have been involved in studies of the effects of light and of sleep shifts on neuroendocrine functioning and various circadian rhythms (see Project Z01 MH 00450-07 CP) and in studies of the biochemical, physiological, and behavioral effects of desipramine and zymelidine. Recently, greater use is being made of outpatient volunteers as controls in the various studies. In practice this population enables our use of beds to be more cost-effective than is the case with the traditional live-in volunteers. A major new development has been the establishment of a special room in which patients' and normal volunteers' circadian rhythms can be studied in isolation from external time cues which

normally mask and entrain the rhythms. A computer-based monitoring system is being developed for the temporal isolation facility, and several new projects involving the facility are underway.

A second major development is the incorporation of a sleep monitoring facility into the ward. Transfer of the sleep laboratory to our inpatient unit will facilitate nursing and medical oversight of subjects whose sleep is recorded, and will foster the integration of sleep physiology and circadian rhythm approaches to research in affective illness.

Significance to biomedical research and to the program of the Institute:

By their very nature the projects of the Clinical Research Unit are designed to enlarge our understanding of the causes and treatment of affective disorder. These projects are distinguished by their attempts to develop new types of antidepressant treatment modalities, such as drugs with relatively specific effects on individual neurotransmitter systems, and novel non-pharmacological approaches involving manipulations of the sleep-wake cycle and the light-dark cycle. Temporal isolation experiments may yield information about fundamental causes of some of the disturbances present in affective illness.

Proposed course:

In the future, clinical studies on the research unit will be organized in such a way as to expand and integrate studies of sleep and biological rhythms in affective disorder. To facilitate this process, the sleep laboratory will be moved to 4-West and merged with the Branch. Facilities for temporal isolation studies of circadian rhythms will be upgraded.

A new project involving hypermetabolic doses of thyroid to treat rapid cycling manic-depressives is envisaged. These patients, along with a large new group of seasonal depressives will continue to be a focus of research.

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Rosenthal, N.E. and Goodwin, F.K.: The role of the lithium ion in medicine. Ann. Rev. Med., Vol. 33, 555-568, 1982.

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|---|--|--|-----------------|---------------------------|---------|--------------------------|-------|---------|-------------|----------------------------|--|------------------|--------------------|---------|--------------|-----------------|--------------|------------------|-----------|---------|------------------|-------|----------|-------------|---------------------|----------|-----------------|---------------------|----------|-------------------|--------------|----------|---------------------|------------------------------|---------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00450-08 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Biological Rhythms in Affective Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Thomas Wehr</td> <td style="width: 33%;">Chief, Clinical Res. Unit</td> <td style="width: 33%;">CP/NIMH</td> </tr> <tr> <td>OTHER: Frederick Goodwin</td> <td>Chief</td> <td>CP/NIMH</td> </tr> <tr> <td>Alfred Lewy</td> <td>Assoc. Prof., Univ. Oregon</td> <td></td> </tr> <tr> <td>Norman Rosenthal</td> <td>Staff Psychiatrist</td> <td>CP/NIMH</td> </tr> <tr> <td>Gerard Groos</td> <td>Visiting Fellow</td> <td>Univ. Leiden</td> </tr> <tr> <td>Richard Kronauer</td> <td>Professor</td> <td>Harvard</td> </tr> <tr> <td>Theodore Colburn</td> <td>Chief</td> <td>RSB/NIMH</td> </tr> <tr> <td>Bruce Smith</td> <td>Biomedical Engineer</td> <td>RSB/NIMH</td> </tr> <tr> <td>William Vaughan</td> <td>Computer Programmer</td> <td>RSB/NIMH</td> </tr> <tr> <td>Lawrence Tamarkin</td> <td>Staff Fellow</td> <td>IRP/NIMH</td> </tr> <tr> <td>J. Christian Gillin</td> <td>Chief, Unit on Sleep Studies</td> <td>BP/NIMH</td> </tr> </table> | | | PI: Thomas Wehr | Chief, Clinical Res. Unit | CP/NIMH | OTHER: Frederick Goodwin | Chief | CP/NIMH | Alfred Lewy | Assoc. Prof., Univ. Oregon | | Norman Rosenthal | Staff Psychiatrist | CP/NIMH | Gerard Groos | Visiting Fellow | Univ. Leiden | Richard Kronauer | Professor | Harvard | Theodore Colburn | Chief | RSB/NIMH | Bruce Smith | Biomedical Engineer | RSB/NIMH | William Vaughan | Computer Programmer | RSB/NIMH | Lawrence Tamarkin | Staff Fellow | IRP/NIMH | J. Christian Gillin | Chief, Unit on Sleep Studies | BP/NIMH |
| PI: Thomas Wehr | Chief, Clinical Res. Unit | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| Richard Kronauer | Professor | Harvard | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Theodore Colburn | Chief | RSB/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| William Vaughan | Computer Programmer | RSB/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lawrence Tamarkin | Staff Fellow | IRP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| J. Christian Gillin | Chief, Unit on Sleep Studies | BP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Research Services Branch, NIMH; Unit on Sleep Studies, Biol. Psych. Br. NIMH; Albert Einstein Medical School, New York; Harvard University, Cambridge; University of Leiden, Netherlands; Intramural Research Program, NICHD. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Psychobiology Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) Basic research in biological rhythms can be related to affective illness with respect to (1) the inherent cyclicity of the illness (itself a type of biological rhythm) and (2) the involvement of disturbed circadian rhythms (24-hour cycles) in its pathophysiology. This project is designed to explore these connections by interrelating behavioral, physiological and biochemical changes observed (1) through the cycle of the illness, and (2) through the course of the 24-hour day. Results to date indicate (1) in depression the timing of circadian rhythms is abnormally early (phase-advanced) relative to the day-night cycle, (2) some depressives' circadian rhythm phase-advance and mood disturbances can be temporarily corrected by shifting their sleep period 6 hours earlier than usual; (3) animal studies in our laboratory indicate that the two major classes of antidepressant drugs may act by slowing the intrinsic rhythm of circadian pacemakers causing a corrective delay in depressives' abnormally advanced circadian rhythms; (5) in mania the 24-hour sleep-wake cycle lengthens dramatically to 48 hours and escapes from its normal 1:1 mode of coupling to the day-night cycle; (6) patients with recurring winter depressions improve after their winter "day" is lengthened with bright artificial light. Principles drawn from basic research in biological rhythms thus have led to two novel non-pharmacological treatments of depression. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Circadian rhythms are near-24-hour patterns of variation in behavior and physiology. They are generated by a clocklike pacemaker in the brain and are synchronized with the external day-night cycle by environmental stimuli that have the properties of time cues.

There is considerable evidence that time-keeping by a biological clock is abnormal in depressive illness and that this abnormality may play an important causal role in the illness.

This project is designed (1) to document the nature of circadian rhythm disturbances in affective illness; (2) to evaluate the effect of direct manipulations of circadian rhythms (shifts in the timing of the sleep-wake and light-dark cycles) on the course of the illness; and (3) to investigate the effects of antidepressant drugs on the circadian rhythm pacemaker.

Behavioral parameters studied involve both nurses' ratings and self-ratings, as well as motor activity monitoring using a novel nontelemetric solid state, computer-based device developed at NIH. Physiological parameters include EEG sleep studies and temperature; biochemical variables include melatonin, cortisol, thyroid stimulating hormone (TSH), growth hormone (GH), and prolactin (PL). These data are evaluated by time series analyses and other methods in order to characterize circadian rhythms.

We can describe the findings in these studies under several headings:

1. Circadian rhythms are 24-hour cycles of biological functions which are driven by neural pacemaker cells in the hypothalamus. These rhythms have characteristic timing and waveforms which are genetically determined and homeostatically conserved. For example, body temperature rises each day to an evening peak, then declines to a late night low. We have studied the timing or phase-position of circadian rhythms in depressives compared with normals and compared with themselves in non-depressed states. Previously we found that the timing of a variety of circadian rhythms is abnormally early in depressives. This work has been extended and confirmed with additional data on REM sleep, rectal temperature and plasma cortisol all indicating a phase-advance.
2. We have continued to study bipolar patients longitudinally through one or more manic-depressive cycles. In these patients the timing of the circadian temperature rhythm became progressively earlier until the patient switched into depression; it then became progressively later until the patient switched out of depression. The range of these shifts was 5 to 12 hours. The finding of a maximal phase-advance at the switch into depression is consistent with our finding that circadian rhythms are phase-advanced in depressives compared with controls, a conclusion also consistent with our systematic review of the literature. Our interpretation that extensive sleep EEG findings in depression represent a phase-advance has stimulated considerable interest among sleep researchers as well as circadian physiologists. In patients in whom sleep EEG was studied longitudinally we continue to find parallel state-dependent changes in the timing of the temperature rhythm and REM sleep abnormalities.

For studies of temperature we have continued to use a self-contained, solid-state rectal temperature recorder which patients wear on their belt, and which monitors temperature continuously. Data from these instruments can be fed directly into a computer for subsequent plotting and analysis. A large scale cross-sectional study of circadian temperature rhythms and EEG-monitored sleep in depressed outpatients is underway. To date we have measured core body temperature in this manner in 20 depressed patients and ten normal subjects. Normal subjects' rectal temperature minimum occurred in the later half of the sleep period. On many nights, rectal temperature minima occurred in the first half of the sleep period in those patients (N=10) who also exhibited the typical patterns of REM sleep distribution seen in endogenous depression. Patients whose temperature minima fell in the latter half of the sleep period had normal REM sleep patterns. These results lend further support to the hypothesis that REM sleep abnormalities in endogenous depression are partly the result from a phase-advance of the circadian pacemaker that controls core temperature.

2. We have hypothesized that the phase-advance of circadian rhythms in relationship to sleep is a critical factor in establishing and maintaining the depressive state. Depriving a patient of sleep during the entire night often induces a dramatic, but short-lived, remission of the depression, suggesting that disrupting the abnormal relationship of sleep to circadian rhythms may be therapeutic. The early timing of circadian rhythms in relationship to sleep could be corrected if depressives' sleep schedule were shifted correspondingly earlier. We had previously studied two patients in whom shifting the onset of sleep from 11 PM to 5 PM produced a remission which lasted two weeks. Following relapse, the patient's sleep was again shifted 6 hours earlier, producing a second remission. In two patients, EEG and rectal temperature were measured two weeks before and one week during the shifted sleep schedule. On the normal 11 PM to 7 AM sleep schedule, temperature minima and REM sleep occurred abnormally early, near the beginning of the sleep period. When the sleep period was shifted 6 hours earlier (5 PM to 1 AM) temperature minima and REM sleep occurred near the end of the sleep period -- a normal pattern. On the second day of the shifted sleep schedule the patient switched out of depression. When the patients eventually returned to a habitual 11 PM to 7 AM sleep schedule they relapsed again into depression. The results of these preliminary experiments suggest that the circadian rhythm abnormalities in depression are not merely epiphenomena but play a causal role in the illness.

Recently three more patients were studied. Two had complete antidepressant responses to the procedure, one did not. One responder, the only patient who received antidepressant drugs, sustained her response to the phase-advance procedure. This result raises the possibility that phase-advance treatment might prove useful in converting drug non-responders to responders and that drugs might help to sustain the antidepressant effect of the phase-advance treatment.

3. Definition of phase, amplitude, and levels of circadian rhythms requires techniques and instruments that permit around-the-clock continuous measurement of relevant variables. Longitudinal studies in patients require that these measurements be carried out day after day for months at a time. Fortunately, the development of a small solid-state instrument by Theodore Colburn and his associates at the Research Services Branch has made it possible to obtain

continuous longitudinal measurements of one variable: motor activity. We have studied the behavioral activity-rest (sleep-wake) cycle for many months in 17 rapidly cycling manic-depressive patients. Our principal finding continues to be that the sleep-wake cycle lengthens from its usual 24-hour period to 48 hours at the onset of mania. Sometimes three or more double length sleep-wake cycles were observed to occur in succession. We infer that the driving oscillator of the sleep-wake cycle markedly slowed its rate of oscillation and escaped from the primary (1:1) to the secondary (1:2) mode of entrainment to the day-night cycle. Similar lengthening of the period of the sleep-wake cycle and shift into and out of the secondary coupling mode also occurs in normal subjects during free-running in isolation experiments. In these normals, the sleep-wake cycle lengthens, escapes from 1:1 coupling with the free-running 25-hour cycles of the circadian temperature rhythm and recouples in a 1:2 mode; thus, the resulting 50-hour sleep-wake cycle locks onto every other 25-hour temperature cycle. When this occurs, normal subjects may remain awake for 30 hours or more with no subjective need for sleep. Manics, too, may stay awake the entire night with no perceived need for sleep.

There are several possible causes of these abnormally long sleep-wake cycles during mania. One possibility is that the intrinsic rhythm of a driving oscillator of the sleep-wake cycle is abnormally slow and thus cannot consistently keep up with the 24-hour rhythm of the day-night cycle, resulting in periodic 48-hour sleep-wake cycles when the coupling shifts from 1:1 to 1:2.

With regard to this possibility, our animal studies indicate that antidepressant drugs may slow the intrinsic rhythm of oscillators which drive circadian rhythms. This slowing could be expected to precipitate abnormal slowing of the sleep-wake oscillator, and might slow the oscillator sufficiently to induce the 48-hour sleep-wake cycle seen at the onset of mania.

In order to investigate the causal implications of these findings, 12 rapidly cycling manic-depressive patients were asked to simulate a 48-hour sleep-wake cycle by remaining awake for 40 hours (one night's total sleep deprivation). Most of the patients switched out of depression into manias, some of which last several weeks. This finding indicates that the abnormal 48-hour sleep-wake cycles that occur when patients spontaneously switch out of depression into mania may play a causal role in the switch process.

Lithium can prevent switches into mania. Therefore, we studied the effects of lithium on the responses of 5 of these patients to total sleep deprivation. Lithium appeared to prevent switches out of depression into mania after sleep deprivation.

Many confounding experimental variables are present during sleep deprivation. For example, during sleep deprivation and during spontaneous 48-hour sleep-wake cycles, patients are exposed to light at a time when they would ordinarily be in the dark, asleep. There are several reasons to consider that light may be an important environmental factor in affective illness (see below). We found that five patients responded equally well to sleep deprivations carried out in a very bright light (3000 lux) and in almost complete darkness (1 lux). This important negative finding establishes that light is not

necessary for the antidepressant effect of sleep deprivation (although we did find that patients were slower to relapse after light than after dark sleep deprivations).

4. In population statistics and in individual cases depression and mania show seasonal patterns of occurrence. Seasonal rhythms in animals depend on the ability of a circadian clock to measure changes in the daily photoperiod (interval between dawn and dusk). Thus, it is possible that seasonally occurring depressions are triggered by changes in the photoperiod. Under the direction of Dr. Norman Rosenthal we are currently exploring this possibility in two ways. First we have initiated a large scale survey of patients with seasonal patterns of depression. To date, over 400 patients have been identified in the Washington, D.C. area. Through questionnaires and interviews, the phenomenology of seasonal depressions was documented. In addition, a longitudinal prospective study of changes in biological and behavioral variables through the course of the year was undertaken, including behavioral ratings, sleep EEG studies, motor activity recordings, temperature measurements, and selected endocrine tests.

Thirty patients and 9 normal controls were studied intensively at different times of year. Our goals were (1) to characterize the syndrome; (2) to determine to what extent seasonal depressives resemble conventional endogenous depressives with regard to various biological markers and diagnostic instruments; and (3) to evaluate the effects of light on the course of their illness. Clinically, patients were found to have a mild type of bipolar affective disorder, with hypomania in the spring and summer and hypersomnic, anergic depressions in the winter. An interesting feature was carbohydrate craving and weight gain during their depressions. Carbohydrate metabolism will be a focus of some of the studies during the coming year. Sleep EEG recordings revealed several abnormalities: during winter depressions patients slept more, but had more disturbed sleep, with increased awakenings and a 50% reduction in delta (slow-wave, or deep) sleep. These objective findings accorded well with patients' descriptions of increased sleep and lack of refreshing sleep in the winter. Responses to dexamethasone suppression (DST) and TRH infusion were normal, as has been reported in other depressed outpatients. Eleven patients were treated with light during their winter depressions. Active (bright white) light was compared to "placebo" (dim yellow) light in a random, cross-over design. Patients were exposed to each type of light for three hours before dawn and for three hours after dusk for 2 weeks (equivalent to a June day). All patients experienced moderate to marked improvement under bright white light; responses to dim yellow light were inconsistent.

This spring several hundred new patients in the Washington area were identified. These patients will participate in a second phase of the study designed to determine whether or not the light treatment acts via a photoperiodic mechanism; that is, whether the timing of light relative to circadian phase (or time of day) is critical. A positive finding would link the light treatment to photoperiodic mechanisms which have been well-studied in animal seasonal reproductive rhythms. In this connection, we found a marked seasonal incidence of conceptions of children of outpatients (over 300 children in the sample), the amplitude of which was five times greater than in the normal population. Conceptions occurred twice as often in summer as in winter. In the coming year

we plan to explore this interesting correlate of the mood cycle more intensively.

The light treatment is another example in which application of principles drawn from basic studies of biological rhythms has led to a novel non-pharmacological treatment of affective illness.

Earlier work in the Branch has demonstrated that chronic treatment with either imipramine (a tricyclic antidepressant) or clorgyline (an MAO inhibitor) produced delays in the timing of the rest activity cycle in hamsters and in the phase position of the circadian rhythm in a number of specific binding sites ("receptors") for a variety of neurotransmitters. During the last year, related work has been undertaken in collaboration with Lawrence Tamarkin of the NICHD. It was found that clorgyline (a type A monoamine oxidase inhibitor) and pargyline (a type B MAOI) delays the timing of the activity-rest cycle by several hours in a dose-dependent manner in the hamster. This result has been confirmed in additional studies where the masking effects of light on activity were controlled, and will be published later this year.

6. Our studies of patients living in a normal environment have raised several questions that can only be answered by studying patients whose circadian rhythms are free-running while they are isolated from all external time cues. For example, do phase-advanced circadian rhythms in depressives result from an overly fast intrinsic rhythm of the circadian pacemaker? We are attempting to explore these and other questions by establishing conditions suitable for free-running experiments on the 4-West research unit. To date, 2 manic-depressive patients have been studied in such conditions. Elaborate precautions were taken to ensure that patients were deprived of external time cues. Sleep EEG, rectal temperature and motor activity were continuously monitored for 4 weeks. One patient, who had been depressed for six months, switched into mania after 3 days, possibly because the timing of sleep relative to other circadian rhythms was affected by the experiments. As predicted, the circadian pacemaker ran abnormally fast (faster than one cycle/24 hours). Other patients will be studied. In addition, the effectiveness of light as a time cue synchronizing circadian rhythms will be investigated in these facilities in normal subjects. If these experiments are successful, patients' responses to light cues will be investigated.

Significance to Biomedical Research and to the Program of the Institute:

This project has advanced our understanding of the causes and treatment of affective disorder in several respects.

(1) Evidence from our clinical studies indicates that the timing of the sleep-wake cycle relative to the timing of circadian rhythms (i.e., their internal phase relationship) can trigger switches into and out of depression in certain patients. This finding suggests that factors that control phase relationships between different components of the circadian system could be responsible for affective episodes and should be investigated in patients and normal volunteers.

(2) Increasing evidence from our animal studies indicates that antidepressant drugs act directly on biological clocks or pacemakers that control the timing

(phase relationships) of components of the circadian system. These effects are of a type that could be expected to correct circadian rhythm abnormalities observed in patients, and therefore may be a mechanism underlying the clinical action of these drugs. Furthermore the circadian system effects may provide a new animal screening method for the identification of drugs with possible antidepressant effects.

(3) Our studies and the concepts on which they were based have led to two novel, non-pharmacological treatments of depression: phase advance of the sleep-wake cycle for endogenous depression and extension of the photo-period with bright artificial light for recurrent winter depression.

Proposed Course:

In the coming year we propose to extend and expand those studies that have been successful:

(1) A new group of seasonal depressives will be investigated. Our principal goal will be to determine whether the antidepressant light treatment operates through a photoperiod mechanism (i.e., depends on an interaction of light with sensitive phases of the circadian system). Clarification of this point would have practical implications for the design of efficient treatment regimes, and would also establish a link with a rich area of basic research into photoperiodic regulation of behavior in animals.

(2) Only two parameters of the central circadian pacemaker can be measured directly: its period (frequency) and its phase response to stimulations with time cues, such as light. Both parameters can only be measured in special conditions where individuals' circadian rhythms can free-run in isolation from external time cues. In order to increase our understanding of circadian rhythm abnormalities in patients, these pacemaker parameters will be measured in patients who are living in isolation from external time cues. (Two patients have already been studied).

(3) The hypothesis that depression is partly caused by an interaction of sleep with a critical sensitive circadian phase will be further explored in a series of experiments in which the timing of sleeping and waking is manipulated. Further definition of the daily timing of critical events underlying the development of the depression syndrome will provide a temporal focus for the search for possible underlying biochemical and physiological mechanisms.

(4) Our finding that clinically effective antidepressant drugs act directly on the circadian rhythm pacemaker has led us to plan a series of animal experiments designed to identify neurotransmitter mechanisms that may underlie these effects.

(5) In order to facilitate studies of the role of the phase relationship of the sleep-wake cycle to circadian rhythms in the pathophysiology of depression, the Sleep Laboratory under the direction of Wallace Mendelson, M.D., will be merged with the Clinical Psychobiology Branch under the direction of its Acting Chief, Thomas A. Wehr, M.D.

Publications:

1. Wehr, T.A. and Wirz-Justice, A.: Internal coincidence model for sleep deprivation and depression. In Sleep 1980, Koella, W.P. (ed.), Karger, Basel, 1981, pp. 26-33.
2. Wehr, T.A.: Circadian rhythm disturbances in depression and mania. In Rhythmic Aspects of Behavior, Brown, F. and Graeber, R.C. (eds.), Lawrence Erlbaum Associates, Hillsdale, New Jersey, 1981, in press.
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9. Wehr, T.A. and Wirz-Justice, A.: Circadian rhythm mechanisms in affective illness and in antidepressant drug action. Pharmacopsychiatry 15:31-39, 1982.
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Publications:

12. Wirz-Justice, A. and Wehr, T.A.: Uncoupling of circadian rhythms in hamsters and man. In Sleep 1980, Koella, W.P. (ed.), Karger, Basel, 1981, pp. 64-72.
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14. Rosenthal, N.E., Lewy, A.J., Wehr, T.A., Kern, H.E. and Goodwin, F.K.: Seasonal cycling in a bipolar patient. Psychiat. Res., in press, 1982.
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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01851-05 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Melatonin Studies | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 50%;">Alfred J. Lewy</td> <td style="width: 40%;">Staff Psychiatrist</td> <td style="width: 10%;">CP/NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Frederick K. Goodwin</td> <td>Chief</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Thomas A. Wehr</td> <td>Chief, Clinical Research Unit</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Philip W. Gold</td> <td>Chief, Unit on Neuroendocrinology</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>William Z. Potter</td> <td>Staff Psychiatrist</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Sanford Markey</td> <td>Chief, Pharm. Appl. of Mass Spec.</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Irwin J. Kopin</td> <td>Chief</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Lawrence Tamarokin</td> <td>Physiologist</td> <td>IRP/NICHD</td> </tr> <tr> <td></td> <td>David Newsome</td> <td>Staff Ophthalmologist</td> <td>CB/NEI</td> </tr> <tr> <td></td> <td>Ernest Ballintine</td> <td>Chief</td> <td>CB/NEI</td> </tr> </table> | | | PI: | Alfred J. Lewy | Staff Psychiatrist | CP/NIMH | OTHER: | Frederick K. Goodwin | Chief | CP/NIMH | | Thomas A. Wehr | Chief, Clinical Research Unit | CP/NIMH | | Philip W. Gold | Chief, Unit on Neuroendocrinology | CP/NIMH | | William Z. Potter | Staff Psychiatrist | CP/NIMH | | Sanford Markey | Chief, Pharm. Appl. of Mass Spec. | LCS/NIMH | | Irwin J. Kopin | Chief | LCS/NIMH | | Lawrence Tamarokin | Physiologist | IRP/NICHD | | David Newsome | Staff Ophthalmologist | CB/NEI | | Ernest Ballintine | Chief | CB/NEI |
| PI: | Alfred J. Lewy | Staff Psychiatrist | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| OTHER: | Frederick K. Goodwin | Chief | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Thomas A. Wehr | Chief, Clinical Research Unit | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Philip W. Gold | Chief, Unit on Neuroendocrinology | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | William Z. Potter | Staff Psychiatrist | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Sanford Markey | Chief, Pharm. Appl. of Mass Spec. | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Irwin J. Kopin | Chief | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Lawrence Tamarokin | Physiologist | IRP/NICHD | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | David Newsome | Staff Ophthalmologist | CB/NEI | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Ernest Ballintine | Chief | CB/NEI | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH; Intramural Research Program, NICHD; Clinical Branch, NEI. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Psychobiology Branch SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS: 2.7</td> <td style="width: 33%;">PROFESSIONAL: 1.2</td> <td style="width: 33%;">OTHER: 1.5</td> </tr> </table> | | | TOTAL MANYEARS: 2.7 | PROFESSIONAL: 1.2 | OTHER: 1.5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Secretion of melatonin by the pineal gland is cyclically stimulated by a circadian pacemaker in the hypothalamus and is inhibited by environmental light acting on the pacemaker structure. Thus, plasma melatonin can be used as a biochemical measure of the oscillations of a circadian pacemaker and its interaction with the light-dark cycle to which it is entrained. The pineal is also involved in the recognition of and response to seasonal changes in the environmental photoperiod (dawn-dusk interval). Using a gas chromatographic-mass spectrometric (GC-MS) assay developed in collaboration with Dr. S. Markey of the LCS, Dr. Lewy has investigated circadian rhythms, seasonal rhythms, and sensitivity to light in blind persons and in patients with affective disorders. This year, preliminary findings in both populations have been replicated in a larger number of subjects; we found that (1) the timing of blind persons' melatonin circadian rhythms relative to the day-night cycle is markedly abnormal - in some cases the rhythm is no longer synchronized with the day-night cycle but free-runs according to its own intrinsic 25-hour period. (2) Manic-depressive patients are abnormally sensitive to light. The former finding highlights the importance of light in the homeostasis of circadian rhythm phases and the latter finding suggests a possible cause for circadian rhythm phase disturbances in manic-depressive illness. Because of Dr. Lewy's departure the project will be terminated.</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: Our group has been interested in the pineal gland because of its involvement in the organism's responses to light, particularly with regard to the regulation of circadian (near 24-hour) and seasonal rhythms. Clinical studies of the Branch have focused on manic-depressive patients, in whom the timing of circadian rhythms appears to be disordered and in whom manipulations of circadian rhythms may lead to remissions or other clinical state changes. A large number of patients with seasonal depressions have also been studied and preliminary results indicate that they can be treated with manipulations involving exposure to light at critical times of day.

Melatonin secretion occurs during the night. Its nightly secretory rhythm is driven by the suprachiasmatic nuclei, a circadian pacemaker in the hypothalamus. Light acts on this system through retinal projections that reach the hypothalamus through the retinohypothalamic tract. Light suppresses the nocturnal rise in melatonin secretion. Light also normally serves to synchronize (entrain) the oscillations of the circadian pacemaker with the 24-hour day-night cycle. We have used around the clock measurements of plasma melatonin to monitor the oscillations of the circadian pacemaker and its responses (sensitivity) to environmental light.

Methods: Previously, Dr. Lewy, in collaboration with Dr. S. Markey of the LCS, developed a highly sensitive and specific negative ionization gas chromatographic mass spectrometric (GCMS) assay for melatonin. This assay was shown to be capable of measuring the extremely low concentrations of melatonin present in human plasma (1-2 picograms/ml in the daytime) in as little as 1 ml. We previously determined that human melatonin is secreted only at night, as in other species. We also found that humans require much brighter light (1500-2500 lux) to suppress melatonin secretion than other species.

Major Findings this year include:

- (1) Our initial finding that manic-depressive patients are hypersensitive to light have been extended to a larger group of patients. 500 lux, which is insufficient to suppress melatonin secretion in normal subjects, is sufficient to suppress it in manic-depressives. Hypersensitivity to light's effect on the retinohypothalamic tract - suprachiasmatic nuclei complex could explain circadian rhythm phase disturbances in these patients. This explanation will eventually be tested more directly by studying patients' circadian rhythm phase responses to light pulses applied in temporal isolation conditions.
- (2) Because of our interest in the role of light as an external time cue that synchronizes circadian rhythms to the day-night cycle, we have also studied patterns of melatonin secretion in blind persons in collaboration with Drs. Newsome and Ballintine of the National Eye Institute. Our first study revealed that the phases of the melatonin circadian rhythm were markedly abnormal in blind persons. Some of them secreted melatonin only in the daytime, instead of the normal nighttime pattern. In a subsequent longitudinal study we found that a subgroup

of blind persons actually had free-running melatonin circadian rhythms; that is, the rhythm was no longer entrained to the 24-hour day-night cycle, but followed its own nearly 25-hour intrinsic rhythm. Rectal temperature, motor activity and melatonin rhythms have been studied in an additional eight patients during the past year. In four patients there was evidence that circadian rhythms were not normally entrained to the day-night cycle. These results confirm that light plays an important role in the synchronization of human circadian rhythms to the 24-hour day-night cycle, and lends some support to the hypothesis that manic-depressives' circadian rhythm disturbances may be partly related to their abnormal sensitivity to light.

Significance to Biomedical Research and to the Program of the Institute:

Melatonin studies have proven particularly useful in advancing our understanding of the role of environmental light in the regulation of human physiology. Using light-suppression of melatonin secretion we have established that very bright light (> 1500 lux) is necessary to elicit biological responses in humans that occur at very low light intensities in animals. We have used this information to design effective light exposure regimes to treat patients with seasonal depressions. This information will also be used when we investigate the role of light in the entrainment (synchronization) of human circadian rhythms.

Proposed Course:

The melatonin project will be terminated because Dr. Lewy, the principal investigator, has left the NIH. Dr. Lewy will continue to collaborate with members of the Branch, and melatonin-related projects will be subsumed under project Z01 MH 00450-07 CP, biological rhythms in affective illness.

Publications:

Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A., and Rosenthal, N.E.: Manic-depressives may be supersensitive to light. Lancet, i:383-384, 1981.

Lewy, A.J., Wehr, T.A., and Goodwin, F.K.: Sunlight and artificial light have different effects on melatonin secretion in humans. In: Advances in the Biosciences, Night and Shift Work: Biological and Social Aspects, Vol. 30. Reinberg, A., Vieux, N., and Andlaur, P. (Eds.). Pergamon Press, Oxford, pp. 107-108, 1981.

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Lewy, A.J.: Human melatonin secretion I: A marker for adrenergic function. In: Biology of Mood Disorders. Post, R.M., and Ballenger, J.C. (Eds.). Plenum Press, New York, in press.

Lewy, A.J.: Human melatonin secretion II: A marker for the circadian system of man and the effects of light. In: Biology of Mood Disorders. Post, R.M., and Ballenger, J.C. (Eds.). Plenum Press, New York, in press.

Lewy, A.J.: Biochemistry and regulation of melatonin production. In: The Pineal Gland. Reikkinen, R.M. (Ed.). Elsevier/North Holland, New York, in press.

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Lewy, A.J.: Effects of light on the human circadian system. In: Biological Rhythms and Depression, Advances in Sleep Research. Weitzman, E. (Ed.). Spectrum Publ., New York, 1982.

Lewy, A.J.: Human melatonin secretion and adrenergic function. In: The Pineal Gland and Its Endocrine Role. Fraschini, F. (Ed.). Plenum Press, New York, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00447-13 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| TITLE OF PROJECT (80 characters or less) Amine Neurotransmitters and Metabolites in Mental Illness | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 60%;">Frederick K. Goodwin</td> <td style="width: 30%;">Chief</td> <td style="width: 10%;">CP/NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Rex W. Cowdry</td> <td>Chief, Outpatient Unit</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Markku Linnoila</td> <td>Staff Psychiatrist</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>William Z. Potter</td> <td>Chief, Unit on Clin. Psychopharm.</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Thomas A. Wehr</td> <td>Chief, Clinical Research Unit</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Robert Post</td> <td>Chief, Clinical Research Unit</td> <td>BP/NIMH</td> </tr> <tr> <td></td> <td>Edna Gordon</td> <td>Chief, Unit on Clinical Biochem.</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Daniel van Kammen</td> <td>Chief, Unit on Neuropsychopharm.</td> <td>BP/NIMH</td> </tr> <tr> <td></td> <td>David Rubinow</td> <td>Staff Psychiatrist</td> <td>BP/NIMH</td> </tr> <tr> <td></td> <td>Michael Ebert</td> <td>Chief, Section on Exp. Ther.</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>David Jimerson</td> <td>Staff Psychiatrist</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Dennis Murphy</td> <td>Chief</td> <td>CN/NIMH</td> </tr> <tr> <td></td> <td>David Pickar</td> <td>Chief, Unit on Studies of Drug Abuse</td> <td>BP/NIMH</td> </tr> <tr> <td></td> <td>Gerald L. Brown</td> <td>Staff Psychiatrist</td> <td>BP/NIMH</td> </tr> </table> | | | PI: | Frederick K. Goodwin | Chief | CP/NIMH | OTHER: | Rex W. Cowdry | Chief, Outpatient Unit | CP/NIMH | | Markku Linnoila | Staff Psychiatrist | CP/NIMH | | William Z. Potter | Chief, Unit on Clin. Psychopharm. | CP/NIMH | | Thomas A. Wehr | Chief, Clinical Research Unit | CP/NIMH | | Robert Post | Chief, Clinical Research Unit | BP/NIMH | | Edna Gordon | Chief, Unit on Clinical Biochem. | LCS/NIMH | | Daniel van Kammen | Chief, Unit on Neuropsychopharm. | BP/NIMH | | David Rubinow | Staff Psychiatrist | BP/NIMH | | Michael Ebert | Chief, Section on Exp. Ther. | LCS/NIMH | | David Jimerson | Staff Psychiatrist | LCS/NIMH | | Dennis Murphy | Chief | CN/NIMH | | David Pickar | Chief, Unit on Studies of Drug Abuse | BP/NIMH | | Gerald L. Brown | Staff Psychiatrist | BP/NIMH |
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| | Markku Linnoila | Staff Psychiatrist | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | William Z. Potter | Chief, Unit on Clin. Psychopharm. | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Thomas A. Wehr | Chief, Clinical Research Unit | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Robert Post | Chief, Clinical Research Unit | BP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Edna Gordon | Chief, Unit on Clinical Biochem. | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | David Pickar | Chief, Unit on Studies of Drug Abuse | BP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Gerald L. Brown | Staff Psychiatrist | BP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH; Biological Psychiatry Branch, NIMH; Clinical Neuropharmacology Branch, NIMH. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Psychobiology Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS: 1.7</td> <td style="width: 33%;">PROFESSIONAL: 1.1</td> <td style="width: 33%;">OTHER: 0.6</td> </tr> </table> | | | TOTAL MANYEARS: 1.7 | PROFESSIONAL: 1.1 | OTHER: 0.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Amine neurotransmitters and their metabolites are studied in blood, cerebrospinal fluid (CSF), and urine to assess neurochemical alterations which may be associated with clinical diagnoses, clinical states, particular behavioral or psychopathological dimensions, or treatment with drugs. A high pressure liquid chromatographic assay for monoamine neurotransmitters and their metabolites in blood, urine, and CSF was developed. Two further studies confirm previous reports of an association between low CSF 5-hydroxyindoleacetic acid and impulse dyscontrol, extending this finding to psychopathic murderers and to suicidal individuals with schizophrenia. In studies of urinary metabolites, five different antidepressant treatments, including lithium and electroconvulsive therapy, have been shown to reduce total norepinephrine turnover. Urinary phenylethylamine excretion was elevated in rapid-cycling bipolar patients during delusional episodes. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

The purpose of this project is the direct assessment of the functional state of brain neurotransmitter amines in patients and normal individuals by assay of levels of the amines and their major metabolites in plasma, in urine, and in the cerebrospinal fluid (CSF). The metabolites under study are 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin; homovanillic acid (HVA), the major metabolite of dopamine; and 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3-methoxy-4-hydroxyphenylacetic acid (VMA), the major metabolites of norepinephrine.

The Chief, CPB, serves as the coordinator for CSF studies in the Division; the Unit on Neurochemistry, CPB, provides sample processing and assays (Dr. Markku Linnoila in collaboration with Edna Gordon and Michael Ebert of the LCS); this Branch also provides central data storage and analysis for CSF results. The major collaborating groups for the CSF studies are the Sections on Psychobiology (Dr. Post) and Neuropharmacology (Dr. van Kammen) of the Biological Psychiatry Branch and the Clinical Neuropharmacology Branch (Dr. Murphy). Studies from the Clinical Psychobiology Branch are reported in detail here. Studies from collaborating groups are reported in the annual reports of the groups involved.

Methodological Issues

We have previously employed gas chromatographic-mass spectrometric assays for monoamine metabolites, which can produce relatively sensitive and specific results even at the low metabolite concentrations found in non-probenecid CSF studies. However, GC-MS assays suffer from several drawbacks, including the need for derivatization steps and the relative scarcity of time on the mass spectrometer. Because of these drawbacks, Dr. Markku Linnoila has done extensive work on the development of a high pressure liquid chromatographic (HPLC) method for assaying CSF metabolites. Values obtained using Dr. Linnoila's HPLC method agree closely with those obtained in parallel GC-MS assays, suggesting that the HPLC provides an accurate and available methodology for routine analysis of clinical specimens. Dr. Linnoila has also developed HPLC assays for the neurotransmitters themselves, which, when fully validated, will enable us to measure both neurotransmitters and their metabolites in a single sample using a single methodology, rather than relying on a series of measurements by different collaborators in different laboratories utilizing different methodologies.

The availability of essentially simultaneous measurements of monoamine neurotransmitters and their metabolites using a single methodology opens a new realm of inference, including the study of complex interrelationships between neurotransmitter release, reuptake, and degradation in single neurotransmitter systems and the interrelationships among catecholamine and indoleamine neurotransmitter systems. When these measures of neurotransmitter turnover are combined with data regarding overall functional monoaminergic activity derived from specific neuroendocrine probes and with data regarding peripheral alterations in monoamine receptor sites, integrated studies of monoaminergic function in psychiatric disorders become possible.

Clinical Studies of Monoamine Neurotransmitters and Their Metabolites

In collaboration with other NIMH units, we have continued the exploration of CSF amine metabolites within and between various clinical states and diagnostic groups (affective illness, schizophrenia, schizo-affective disorder, and other disorders such as anorexia nervosa).

The primary new clinical findings are extensions of previous studies associating serotonin metabolism with impulsive or violent behavior. Previous studies from Scandinavia suggest that low levels of CSF 5-HIAA may be associated with violent suicide attempts in both depressed and non-depressed subjects. This finding corresponds to a previous finding reported by our Branch in collaboration with the National Naval Medical Center, suggesting that, among young males with character disorders, low CSF 5-HIAA is relatively strongly associated with a lifetime history of recurrent violent and often impulsive acts. Another study performed by Dr. Lavonne Brown in collaboration with our Branch and the National Naval Medical Center suggests that low CSF levels of 5-HIAA are significantly correlated with high scores on the Pd scale of the MMPI, and are nearly significantly correlated with a lifetime history of violent acts, when studied in a group of individuals with borderline personality disorder. A retrospective chart review of patients with major depressive disorder studied on our inpatient unit over the past eight years suggests that there is a significant correlation between low levels of CSF 5-HIAA following probenecid administration and the occurrence of significant episodes of behavioral dyscontrol at some point during the patient's lifetime. This association was nearly significant both for overt attempts at suicide and for overt acts of violence, and becomes quite significant when suicide and violence are considered as alternative forms of behavioral dyscontrol. Recently completed HPLC assays of monoamine metabolite concentrations in CSF, performed in our Branch by Dr. Linnoila in collaboration with colleagues in Finland, found that psychopathic murderers have low concentrations of 5-HIAA in CSF, in contrast to individuals who commit murder while experiencing paranoid thoughts, who have normal levels of 5-HIAA. Finally, recent HPLC results from our Branch in collaboration with the schizophrenia studies unit at the Clinical Center show that schizophrenic individuals who are suicidal have significantly lower CSF levels of 5-HIAA than those who are not suicidal. These findings suggest that certain biochemical abnormalities may have behavioral significance which cuts across traditional diagnostic boundaries.

Studies of monoamine metabolites in urine have also employed the HPLC methodology. Previously reported findings of high correlations of excretion rates of NE, MHPG, normetanephrine, and vanillylmandelic acid across individuals have been extended to demonstrate further correlations with the excretion rate of tyramine. High urinary phenylethylamine excretion rates are associated with delusional states in rapid-cycling bipolar patients. Perhaps most significant is the finding that five different antidepressant treatments, including lithium and electroconvulsive therapy, all reduce total norepinephrine metabolism in depressed patients, suggesting a common biological effect for apparently dissimilar treatment modalities. Thus, the postulated importance of norepinephrine systems in the pathophysiology and treatment of affective disorders continues to receive indirect support.

Significance to Biomedical Research and to the Program of the Institute:

The major theories about the biological causes of the most prevalent severe psychiatric disorders, depression and schizophrenia, center on monoamine neurotransmitter systems. This project applies sophisticated laboratory assays directly to human studies of monoamine metabolism. Results reported this year confirm previous reports of an association between low levels of the serotonin metabolite 5-HIAA and behavioral dyscontrol, extending this finding to two new groups: suicidal schizophrenics and psychopathic murderers. These consistent findings across diagnostic groups suggest both an underlying biochemical predisposition to acts of dyscontrol and possible treatment approaches involving serotonergic agents such as lithium. Other results expand our understanding of the role of norepinephrine in depression and the possible mechanisms of action of antidepressant treatments, since five very different antidepressant treatments all produce similar effects on total body norepinephrine turnover in depressed patients.

The personal and social costs of depression and disorders of impulse control are great. Insofar as careful clinical research, drawing on basic biochemical techniques, can suggest biological factors in these disorders, specific pharmacologic treatments can be developed and tested in therapeutic trials.

Proposed Course:

With the proposed reorganization of the Intramural Research Program, results previously reported under this project number will be reported in the annual reports of each Branch individually. Except for studies conducted primarily within the Clinical Psychobiology Branch, this procedure is already followed. In particular, studies will be reported in annual reports of the Biological Psychiatry Branch and the Clinical Neuropharmacology Branch. This project number will be discontinued.

Publications:

Potter, W.Z., Muscettola, G., and Goodwin, F.K.: Sources of Variance in Clinical Studies of MHPG. In Maas, J.W. (Ed.): MHPG and Psychopathology. New York, Academic Press, in press.

Linnoila, M., Karoum, F., and Potter, W.Z.: High correlation of norepinephrine and its major metabolite excretion rates. Arch. Gen. Psychiatry, 39:521-523, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01850-05 CP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Clinical Pharmacology of Antidepressants | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: William Potter OTHER: Frederick Goodwin Markku Linnoila Richard Ross Matthew Rudorfer Thomas Wehr Mika Scheinin Wen-Ho Zhang Ming-dao Zhang Elizabeth Lane Anthony Zavadi | Chief, Unit on Clin. Psychopharm. Coordinator for Clin. Psychophar. Chief Staff Psychiatrist Staff Fellow Staff Fellow Chief, Clinical Research Unit Visiting Fellow Visiting Fellow Visiting Fellow Visiting Fellow Guest Worker | CP/NIMH P-T/NIGMS CP/NIMH CP/NIMH P-T/NIGMS CP/NIMH CP/NIMH CP/NIMH CP/NIMH P-T/NIGMS LCS/NIMH |
| COOPERATING UNITS (if any) Pharmacology-Toxicology Program, NIGMS; Laboratory of Clinical Science, NIMH; Division of Special Mental Health Research, NIMH; Cl. Neuropharma. Br., NIMH; Laboratory of Psychol. and Psychopathol., NIMH; Biol. Psychiat. Br., NIMH. | | |
| LAB/BRANCH Clinical Psychobiology Branch, NIMH | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 3.7 | PROFESSIONAL: 2.9 | OTHER: 0.8 |
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| SUMMARY OF WORK (200 words or less - underline keywords) Studies on the clinical pharmacology of biochemically specific antidepressants produce both therapeutic advances and increased knowledge concerning the biological basis of affective illness. Pharmacokinetic studies have focused on hydroxylation as the most clinically relevant metabolic pathway. We find that decreased hydroxylation among such genetically distinct populations as the Chinese may explain altered drug response. Aging is associated with decreased renal clearance of active hydroxy metabolites and decreased physiologic but not biochemical responses to tricyclic antidepressants. Further pharmacodynamic investigations reveal that five distinct antidepressant treatments - ECT, lithium, clorgyline, desipramine and zimelidine, all ultimately reduced the turnover of norepinephrine and/or its major metabolites in man. The biochemical effects, however, are not sufficient for antidepressant action. Low dose clorgyline, a MAO-type A inhibitor, has proved to be a particularly effective antidepressant and specific for the noradrenergic system. Primate experiments support an interpretation that rodent models identifying <u>serotonin</u> as a substrate for MAO-type A cannot be extrapolated to man. | | |

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

| | | |
|---------------------|--|------------|
| Irwin J. Kopin | Chief, | LCS/NIMH |
| Richard J. Wyatt | Chief, | DSMHR/NIMH |
| Dennis L. Murphy | Chief, | CN/NIMH |
| Neal Cutler | Chief, Section on Brain, Aging and Dementia | LN/NIA |
| Farouk Karoum | Research Chemist | DSMHR/NIMH |
| Monte Buchsbaum | Chief, Sect. on Clin. Psychophar. | BPB/NIMH |
| John Nurnberger | Staff Psychiatrist | BPB/NIMH |
| Elliot Gershon | Chief, Section on Psychogenetics | BPB/NIMH |
| Tom Insel | Staff Psychiatrist | CN/NIMH |
| Herbert Weingartner | Psychologist | LPP/NIMH |

On the basis of our preliminary findings of antidepressant-induced changes in norepinephrine turnover in man (MH-01850-04 CP) the last year has seen a major expansion of studies focused on the noradrenergic system using recently developed methods. Clinical research on antidepressant action has been directed to the remarkable finding that low doses of an investigational selective MAO-type A inhibitor, clorgyline, not only has a potent antidepressant effect in bipolar patients but an anti-cycling effect as well. Continual assessment of clinically relevant pharmacokinetic variation following administration of tricyclic antidepressants has been possible by undertaking Chinese/Caucasian cross-racial study using desipramine as a model compound.

During a period of multiple structural changes in the IRP of the NIMH, personnel and project direction have remained relatively constant although planned expansion in areas involving laboratory space or outpatient clinics has been necessarily restricted. Nevertheless, through the ingenuity of the investigators it has been possible to greatly expand the scope of studies. Dr. Markku Linnoila, converted in July, 1982 from an IPA to a staff position, has not only effected the greatly expanded analytical capability of our laboratory but has also solidified a number of highly productive collaborations, especially with Drs. Farouk Karoum and Monte Buchsbaum. Dr. Matthew Rudorfer with the assistance of Dr. Wen-ho and Ming-dao Zhang, the latter beginning work in January, 1982 as the second official fellow under the US-PRC Joint Health Agreement in Mental Health, identified and studied a population of 30 age and sex-matched Chinese and Caucasian volunteers. Dr. Wen-ho Zhang has also developed in the HPLC laboratory to the point where he will be able to direct an analytical facility in Beijing. Dr. Ming-dao Zhang has rapidly mastered techniques of obtaining samples for biochemical analysis from patients and will focus on the use of radio-immunoassays. On the basis of his exceptional level of performance in the laboratory, Dr. Mika Scheinin's visiting fellowship was extended through December 1982. Although primarily involved in basic neurophysiological research during the last year, Dr. Richard Ross has continued to be an important part of clinical studies directed toward the assessment of noradrenergic function in man. Current plans in the area of noradrenergic mechanisms entail extensive collaborations with Dr. Irwin Kopin. Finally, the antidepressant-alcohol interaction study under the direction of Dr. Linnoila has been particularly fruitful thanks to collaborations with Drs. Monte Buchsbaum and Herbert Weingartner.

Assay Development: We have continued work on new liquid chromatography methods for the measurement of MHPG, HVA and 5HIAA and 5HT in biological samples. These assays are distinguished from existing methods by mobile phases which consist of organic amine bases and by unique internal standards 6-fluoro HVA and 6-fluoro 5HT, which were synthesized on the NIH campus at our request. These developments yield a greater accuracy and shorter chromatography time than any existing liquid chromatography assay systems for these substances. In addition, we now can routinely use liquid chromatographic assays for the neurotransmitters dopamine and norepinephrine. The combination of assays allows us to quantitate these substances of interest in small aliquots of human cerebrospinal fluid, plasma or urine and in very small pieces of brain tissue of experimental animals. Interestingly, the only exception is that HPLC systems for MHPG measurement in urine have proved impractical so that studies on this parameter are done with a GC-MS assay.

Pharmacokinetic Studies:

1. Single dose pharmacokinetic predictions of therapeutic drug doses (Z01 MH 01850-01 CP) have proved particularly useful in research in the elderly. Our studies of the aged done in collaboration with Dr. Neal Cutler have depended on the routine use of this technique.
2. We have replicated our finding that the concentration of the active hydroxy metabolite of desipramine, initially identified by our earlier investigations (Z01 MH 01850-02 CP), is elevated in the elderly.
3. Prospective pharmacokinetic studies by Dr. Elizabeth Lane and Dr. Rudorfer employing recently developed pharmacokinetic models have begun to clarify the relative contributions of tricyclic metabolism vs. renal elimination in determining effective drug concentration. Data derived from our just-completed study of desipramine (see below) show that whereas hydroxylation rates are the major determinant of steady-state concentrations of parent tricyclic antidepressants, renal elimination is the critical variable determining the steady-state concentrations of active hydroxy metabolites. Ours is the first demonstration of this and should have general implications for a wide variety of psychoactive and cardiovascular compounds.
4. Both complete five-day blood and urine collections have been made following single dose desipramine in a cross-racial Chinese/Caucasian study with quantitation of parent drug and its conjugated and unconjugated hydroxy metabolite in both tissues. Results of those studies establish that Chinese have significantly lower rates of hydroxylation than Caucasians, a fact which could explain the lower doses of tricyclic antidepressants as well as beta blockers needed in Chinese. This study is, to our knowledge, the only one in which true hydroxylation clearances can be calculated thereby permitting detailed analysis to see if drug hydroxylation fits a simple genetic model. Preliminary pharmacogenetic studies using debrisoquine as a model compound have suggested that slow hydroxylation is more frequent in Orientals based on urinary ratios which provide only a rough estimate. We have undertaken studies to show that our results with desipramine are generalizable by administering debrisoquine to selected subjects from our previous study. The cross-racial study has also been noteworthy in its efficient use of volunteers such that only a nine month period has been required from the recruitment of subjects to completion of a definitive study.
5. The availability of cerebrospinal fluid from patients receiving antidepressant medications has permitted us to continue direct measurements of free drug and provide the most definitive statements concerning unbound drug parent in the central nervous system under steady-state condition. Our findings with zimelidine provide direct evidence of the unreliability of standard in vitro methods for measuring free drug concentration.

Clinical and Biochemical Effects of Antidepressants:

1. Our earlier finding that zimelidine, an experimental antidepressant, specific for serotonin uptake inhibition does not precipitate mania or rapid cycles in bipolar depressed patients (as contrasted to desipramine) has been

supported by world-wide experience in over 2000 patients. If zimelidine proves to be an effective antidepressant in such patients a major therapeutic advantage will result. This study is just beginning in collaboration with Drs. Nurnberger and Gershon of the BPB following delays in receiving clearance to use zimelidine plus lithium.

2. We previously showed that both the acute and chronic effects of desipramine were the same and were most consistent with increased adrenergic function. Parameters which supported this were the pattern of pulse, blood pressure and EKG changes. In collaboration with Dr. Neal Cutler of the National Institute of Aging, we have extended our studies on the elderly. Surprisingly, we find that elderly patients do not show the same increase of pulse and blood pressure as younger subjects having similar increases of norepinephrine. These findings are consistent with reports that post-synaptic beta receptors are decreased in the elderly.

3. In a series of parallel studies we have examined the effects of five distinctly different antidepressant treatments in bipolar and unipolar patients on whole-body norepinephrine turnover. These were based on our preliminary finding that both desipramine and zimelidine appeared to reduce norepinephrine turnover (MH 01850-04 CP).

During the last year we have measured norepinephrine and its major metabolites using a GC-MS assay with Dr. Farouk Karoum before and after treatment with ECT, lithium and clorgyline (a selective MAO-A inhibitor) as well as desipramine (a selective norepinephrine uptake inhibitor) and zimelidine (a serotonin uptake inhibitor). All treatments reduce one or more of the noradrenergic parameters apparently independent of clinical effects. These findings provide substantial support for the hypothesis that effects on the noradrenergic system are ultimately involved in the antidepressant action of all treatments. They are also consistent with basic studies showing either reduced beta receptors and/or isoproterenol stimulated cyclic AMP following antidepressant treatments. A corollary of our findings is that these biochemical effects are not sufficient for antidepressant effect. Furthermore, in all cases where relevant, we have been able to control for pharmacokinetic variance lending further strength to our findings.

4. As part of our effort to find new ways of treating rapid-cycling manic depressive patients we have expanded our clinical studies of low dose clorgyline as a unique and safe treatment. Eleven out of twelve treatment-resistant (i.e. not responding to lithium + standard antidepressants) patients have shown remarkable improvement. In two cases, complete remission of a previously intractable illness has been achieved. Biochemical studies indicate that even in low doses maximum MAO-A inhibition can be achieved without affecting platelet MAO. This discrimination of biochemical and neurophysiologic effects from clinical outcome and MAO-A inhibition has important theoretical and clinical implications. In collaboration with Drs. Murphy and Insel we find that low dose clorgyline produces immediate MAO-A inhibition in monkeys (based on measurements of CSF parameters) selective for the noradrenergic and not the serotonergic system. These findings suggest that MAO specificity studies based on rodent models are not applicable to primates.

5. As part of the biochemical assessment of clorgyline effects, Dr. Linnoila found that subjects with elevated phenylethylamine (PEA) respond poorly to all treatments, a finding which we have extended. Furthermore, drugs which increase PEA frequently, if not inevitably, lead to poor response in manic-depressive patients. Interestingly, low dose clorgyline does not increase PEA. These findings are consistent with the formerly postulated role of PEA as an "endogenous amphetamine" and may provide a means of selecting patients for treatment. We have been able to control at least the peripheral formation of PEA by administration of the decarboxylase inhibitor, carbidopa. We are excited by our initial trial of carbidopa which has been shown to significantly improve a previously totally refractory patient who was found to show markedly elevated excretion of urinary PEA.

6. Efforts to understand the influence of the major classes of psychoactive drugs on psychomotor function have yielded surprising results. It has been possible to distinguish acute effects on psychomotor function of amitriptyline, desipramine and zimelidine alone and in combination with alcohol. As expected, amitriptyline alone impaired performance across a number of variables and in combination with alcohol produced dramatic transient deterioration of functions. Desipramine had far less marked effects but in the same direction. Zimelidine, however, not only did not impair performance but appeared to improve certain aspects of learning and reverse (or prevent) effects of alcohol. New studies with chronic zimelidine are underway to assess the ability of this new drug to improve certain aspects of learning. Of particular interest is that we previously found that zimelidine was unique in its ability to increase vasopressin in patients (MH 01850-04 CP). Studies will also soon begin on zimelidine's effects in the elderly in collaboration with Dr. Neal Cutler.

Significance to biomedical research and to the program of the Institute:

These combined investigations using systematic application of new quantitative methodologies have permitted us to separate actual biochemical effects of antidepressant treatments in patients from those that have been speculated to be important on the basis of preclinical studies. Knowledge of the biochemical specificity of action has enabled us not only to improve the utilization of standard drugs but also to find new and promising therapeutic investigational compounds. Our research therefore both increases our understanding of the biochemical components of one of the two major psychiatric illnesses and provides immediate therapeutic advances.

Proposed Course:

We have been able to answer most of our major pharmacokinetic questions and will concentrate our efforts on developing the most biochemically specific antidepressant treatments, particularly in relation to their effects on the noradrenergic and serotonergic systems.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00452-07 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Neuroendocrine Studies of Major Psychiatric Disorders | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Current studies concentrate on comprehensive tests of hypothalamic-pituitary function and on the measurement and administration of specific behaviorally active <u>peptides</u> or <u>peptide analogs</u> . Major findings this year are: Drug-free bipolar patients show abnormalities in the osmoregulation of plasma arginine vasopressin (AVP) which are significantly correlated with levels of AVP in cerebrospinal fluid (CSF). Levels of CSF <u>somatostatin</u> are significantly reduced in unipolar and bipolar depressed patients compared to controls. <u>Oxytocin</u> (OT) is routinely present in the CSF of normal men and women in similar amounts, and the levels of CSF OT are systematically altered in subgroups of psychiatric patients with primary affective disorder and anorexia nervosa. In the intact and stalk-sectioned cynomolgus macaque, peripheral administration of the newly-sequenced corticotropin releasing hormone (CRH) elicits the following physiological responses typically associated with the stress reaction in primates: cortisol secretion (with an Ed ₅₀ between 0.1 µg/kg and 1.0 µg/kg), growth hormone and prolactin secretion, and tachycardia. The metabolic clearance rate of CRH in primates is exceedingly slow and its plasma half-time is longer than for any other known endogenous hypothalamic peptide. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

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| George Chrousos | Senior Investigator | DEB/NICHD |
| Edward Oldfield | Senior Attending Neurosurgeon | SN/NINCDS |
| Gary Robertson | Professor of Medicine | Univ. of Chicago |
| Gerry DeFrait | Psychiatrist (Private Practice) | Baltimore |

Project Description:

Several aspects of the symptom complex of the functional psychoses, particularly affective illness, suggest hypothalamic dysfunction. Thus, patients with depression or mania often show disturbances in sleep, altered energy levels, changes in appetite and libido, diurnal variation in symptoms, alterations in the consolidation of memory traces, and changes in reproductive function such as amenorrhea. Interest in the hypothalamic-pituitary (HP) axis has been further stimulated by recent findings that the monoamine neurotransmitters modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones. Thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, these peptides and hormones may have specific receptors in brain and have been shown to affect the brain neurotransmitter systems. Several of these hormones have also been shown to be responsible for specific behavioral effects.

Several neuroendocrine strategies are currently in use: (1) direct measurement in CSF and in plasma of behaviorally active peptides during the basal state and/or following stimulation according to verified stimulation paradigms; (2) administration of various hypothalamic releasing factors to test responses of the HP axis, and to elucidate patterns of monoaminergic disturbance and neuroendocrine dysfunction; (3) effects of psychoactive drugs on HP function and on the levels of behaviorally active peptides; (4) assessment of the temporal organization of neuroendocrine function; and (5) assessment of the relationship between neuroendocrine function and sleep. For purposes of comparison and possible differential diagnosis, normal subjects, patients with affective illness, schizophrenia, anorexia nervosa and Cushing's disease are studied. We are also involved in studying the neurobiology of several neuroendocrine diseases, including Cushing's syndrome and Kallman's syndrome, and in developing clinical means for the differential diagnosis of Cushing's syndrome from depression and for distinguishing hypothalamic from pituitary Cushing's disease.

The Unit on Neuroendocrinology has established an extensive collaborative clinical and laboratory study with the Developmental Endocrinology Branch of the NICHD. Clinically, the project focuses on pathophysiological analogies between depression and Cushing's syndrome and on behavioral and endocrine responses to the recently sequenced corticotropin releasing hormone. In the laboratory, we have developed radioimmunoassays for a variety of peptide hormones, including ACTH, β -endorphin, alpha MSH, vasopressin, oxytocin, and CRH. This project will be discussed in detail in section 3 of this report.

1. Studies of behaviorally active central nervous system (CNS) peptides:

a. We are continuing comprehensive investigation of arginine vasopressin (AVP) function, concentrating on studies of CSF AVP, the plasma AVP response to hypertonic saline, and the cognitive and behavioral responses to vasopressin analog administration. We have also initiated a series of studies to examine non-osmolar stimuli to plasma secretion of AVP to directly evaluate neurohypophyseal function.

We have replicated our previous findings that CSF AVP is significantly lower

in non-psychotic drug-free bipolar depressed patients compared to manic subjects or controls. This replication represents an increase in the total number of drug-free patients studied from 30 to 48. We have also further demonstrated that alterations in AVP function in affective illness seem limited to bipolar patients while AVP function in unipolar patients seems intact. Thus, we have noted that in bipolar patients studied longitudinally CSF AVP is significantly lower in the depressed phase than when patients are restudied after recovery; this finding is not noted in the unipolar group. Moreover, defects in the osmoregulation of plasma AVP seem restricted to the bipolar group; bipolar patients in the depressed phase show a subnormal response to hypertonic saline infusion, secondary either to an absolute deficiency in the AVP response or to a pathological advance in the threshold for AVP secretion to the osmotic stimulus, with the result that most points fall below the normal range. In drug-free bipolar patients a positive correlation was noted between the level of AVP in the CSF and the sensitivity of osmoregulation of plasma AVP, suggesting that defects in the two compartments are related.

Lithium and carbamazepine each influence the sensitivity of osmoregulation in the direction opposite to their effects on the renal AVP receptor. These findings suggest the presence of a non-osmolar signal which integrates changes in AVP secretion or actions. Other drugs which influence the sensitivity of osmoregulation are zimelidine and pimozone.

We have further replicated our previous finding noted in 12 drug-free schizophrenic patients showing lower CSF AVP levels compared to controls. This finding, now shown in a total of 23 drug-free schizophrenic subjects, shows that the level of CSF AVP is significantly lower in subjects regardless of clinical status. No systematic alterations in osmoregulation was noted in schizophrenic subjects.

The osmoregulation of arginine vasopressin (AVP) secretion into plasma is consistently abnormal in chronically underweight patients with anorexia nervosa. These abnormalities occur in two forms: most commonly, the secretion of AVP is erratic, so that in contrast to normals, the levels are totally divorced from osmotic influences; less often, the secretion of AVP is sensitive to but grossly subnormal for the levels of plasma osmolality. These defects do not improve immediately after return to ideal body weight. However, in most of the patients studied six months to two years after recovery, all aspects of osmoregulation had normalized. Abnormalities in the osmoregulation of plasma AVP are almost always associated with abnormalities of AVP secretion into the cerebrospinal fluid (CSF). The latter manifests as an absolute increase in CSF AVP and/or a reversal of the normal CSF/plasma AVP ratio. The pathophysiologic consequences of the plasma and CSF defects remain to be defined. Of particular interest are their relationship to subtle abnormalities in regulation of water balance in anorexia nervosa and the putative role of AVP as a neuromodulator capable of influencing cognition.

Because of the cognitive properties attributed to AVP, we also studied the effects of DDAVP administration on cognition in patients with Alzheimer's disease. Such patients show failure to access and use previously learned knowledge (semantic memory) and have difficulty in encoding and processing

ongoing events. DDAVP produced cognitive enhancements in these patients through arousal related to facilitation of these semantic memory structures analogous to effects noted previously in depressed patients and control subject.

b. CSF oxytocin - We have noted for the first time that oxytocin, a structural analog of AVP, is routinely present in the CSF of both male and female subjects. There were no significant differences in the level of CSF OT between men and women, a result that is compatible with findings in experimental animals that estrogen does not influence the secretion of OT into the CSF, in contrast to its effects on the levels of OT secreted into plasma. In psychiatric patients, we have replicated our previous preliminary finding that CSF OT is significantly lower in drug-free manic patients compared to drug-free bipolar depressed patients or controls. OT is also significantly lower in anorexic patients studied during the chronically underweight state compared to when these patients are restudied 3-5 weeks after correction of the weight loss.

c. CSF somatostatin - CSF somatostatin levels were studied in collaboration with Dr. David Rubinow, Dr. Robert Post, and Dr. Seymour Reichlin. CSF somatostatin is significantly lower in a large group of drug-free unipolar and bipolar depressed patients compared to controls. Several psychoactive drugs, including carbamazepine and zimelidine, significantly influenced the level of somatostatin in CSF. The significance of lower somatostatin in the CSF of depressed patients with respect to alterations in hypothalamic-pituitary-thyroid function and responses to TRH in patients with primary affective disorder, is currently being actively investigated.

d. Neuroendocrine effects of opiate agonists and antagonists: We have previously reported that intravenous methadone administration diminishes plasma cortisol secretion in depressed patients. We now note that methadone also releases prolactin and vasopressin in depressed patients, but has no effect on growth hormone and gonadotropin secretion acutely. Naloxone has no effect on the AVP response to hypertonic saline or the TSH or prolactin response to TRH. We are currently studying neuroendocrine effects of opiate administration in both Cushing's disease and depression. To date, we have shown that morphine administration blunts the cortisol response to insulin-induced hypoglycemia in patients with Cushing's disease and control subjects; however, in contrast to normals, morphine does not lower basal cortisol levels nor the circadian-induced rise in corticosteroid secretion. We are currently administering morphine sulfate to depressed patients to see if the corticosteroid response in depression differs from that seen in Cushing's disease, thus raising the possibility of a differential diagnostic test to distinguish the hypercortisolism of depression from that of Cushing's disease.

2. Dynamic tests of monoaminergically regulated HP systems:

We continue to evaluate the neuroendocrine responses to a three-hour dopamine infusion in psychiatric patients. In collaboration with Dr. Gerry DeFraites, we have previously noted exaggerated gonadotropin responses but normal prolactin responses to dopamine infusion in drug-free schizophrenic patients, a finding that suggests an increased sensitivity of some hypothalamic dopamine receptors. Methodologically, the results of this study also suggests that the

gonadotropin rather than prolactin responses represent a usable neuroendocrine marker to assess the functional activity of median eminence dopamine receptors. Preliminary data suggest that the growth hormone response to dopamine infusion in schizophrenia is also exaggerated during the drug-free period. There is also an increased growth hormone response to dopamine in patients studied 3 weeks after neuroleptic withdrawal, a result which suggests that our findings in "drug-free" schizophrenic patients may reflect increased sensitivity of dopamine receptors due to neuroleptic withdrawal.

Drug-free patients with anorexia nervosa show blunted gonadotropin responses to sustained dopamine infusion. Metoclopramide infusion and naloxone infusion, both of which elevate gonadotropin secretion in patients with secondary hypothalamic amenorrhea do not appear to influence gonadotropin regulation in patients with anorexia nervosa, presumably because of refractoriness of unprimed gonadotrophs. We are currently pursuing studies that examine the interface between endogenous opiate systems and dopamine in anorexia nervosa.

3. Collaborative studies of the neurobiology of clinical neuroendocrine disease and the neuroendocrinology of psychiatric illness:

We have developed an extensive collaborative project examining common neuroendocrine and neurobiological aspects of depression and Cushing's disease. Subjects with each of these disorders have been undergoing parallel work-ups, including lumbar puncture, neuroendocrine challenge paradigms, sleep and temperature monitoring, average evoked response, and extensive neuropsychiatric testing. An important clinical focus will be on applications of the newly sequenced corticotropin releasing hormone on studies in humans with neuroendocrine and psychiatric illness. These potential applications include: 1) a new test of pituitary ACTH reserve; 2) a new approach to distinguish hypothalamic from pituitary causes of Cushing's disease and adrenal insufficiency; 3) a new approach to treat chronic pituitary-adrenal suppression; 4) a new treatment for Cushing's disease (if continuous infusion of CRH inhibits the pituitary as does continuous infusion of LHRH; 5) an approach to evaluate new hormones secreted by the corticotroph, such as the putative adrenal androgen stimulating hormone and aldosterone stimulating hormone; 6) evaluation of differential neuroendocrine responses in subgroups of patients with psychiatric illness versus patients with Cushing's disease and controls; and 7) evaluation of the behavioral and cognitive effects of CRH and comparison of these responses with those obtained with other peptides which possess CRH activity, including vasopressin.

Since CRH acts at the corticotroph to cleave fragments of pro-opiomelanocortin, we have developed radioimmunoassays for the following peptide hormones: ACTH, β -endorphin, alpha MSH and gamma MSH. We have also developed a highly specific and sensitive assay for arginine vasopressin. We are currently in the process of validating antibodies produced against CRH in preparation for extensive studies of this hormone in humans and laboratory animals. We have already begun a series of studies of the effects of CRH on the ICV levels of a variety of peptides and neurotransmitters under the leadership of Dr. Edward Oldfield, a neurosurgeon who has recently joined our group to work full-time on studies of CRH and related peptides.

To explore the physiology of CRH in primates, we determined the plasma cortisol response to graded doses of this peptide in the cynomolgous macaque. We used stalk sectioned monkeys in the dose response studies to avoid interference by endogenous CRH. The animals were stalk sectioned via the transpalatal approach and were maintained on daily injections of synthetic ACTH 1-24 for one week. Synthetic CRH was then given as an IV bolus. Ten doses of CRH (0, 0.1, 0.5, 1, 2, 4, 10, 20, 40 $\mu\text{g/kg}$) were used. CRH stimulated cortisol secretion with an ED_{50} between 0.1 and 1 $\mu\text{g/kg}$ body weight. The peak cortisol response occurred after 15-30 minutes at low doses (0.5-1 $\mu\text{g/kg}$) and after 45-90 minutes at higher doses (2-4 $\mu\text{g/kg}$). CRH also released growth hormone and prolactin at all doses above 1 $\mu\text{g/kg}$. This response occurred not only in the stalk sectioned animals but in an additional series of intact animals as well. In the same animals CRH induced a marked transient increase in heart rate. Thus, peripheral administration of CRH elicited several responses characteristic of the stress reaction in primates including cortisol secretion, growth hormone and prolactin secretion, and tachycardia. Work from other laboratories suggests that the increased heart rate results from a CRH-induced release in catecholamines.

We also determined the metabolic clearance rate and plasma half-life of I^{125}CRH and $\text{I}^{125}\text{Tyr}^0\text{-CRH}$ in cynomolgous monkeys, using the pulse injection and the continuous infusion methods.

Hunter-Bolton reagent was used for iodination of CRF and the chloramine-T method for $\text{Tyr}^0\text{-CRF}$. Gel chromatography was used to separate free iodine (and radioiodinated small fragments) from iodinated CRF in plasma samples. Disappearance of $\text{I}^{125}\text{-CRF}$ or $\text{I}^{125}\text{-Tyr}^0\text{-CRF}$ could be modeled with two components (bi-exponential). Plasma half-life of $\text{I}^{125}\text{-CRF}$ was 17.1 ± 2.44 min (mean \pm SE, $n = 4$) for the fast component and 198 ± 5.3 min for the slow component. The MCR of $\text{I}^{125}\text{-CRF}$ using the pulse injection technique was 0.44 ± 0.06 L/Kg/d, $n = 4$. Continuous infusion of $\text{I}^{125}\text{-CRF}$ gave a MCR of 5.03 ± 0.06 L/Kg/d, $n = 2$. The continuous infusion MCR of $\text{I}^{125}\text{-Tyr}^0\text{-CRF}$ was 2.26 ± 0.03 L/Kg/d, $n = 2$. $\text{I}^{125}\text{/ACTH 1-39}$ MCR was measured as a control using the same technique and gave a MCR of 22.24 ± 3.19 L/Kg/d, $n = 2$. Thus, the plasma half-life of CRF is longer than for all other known hypothalamic peptides. Its metabolic clearance rate is relatively low and is actually less than that of ACTH.

Significance to Biomedical Research and to the Program of the Institute:

The work of the Unit on Neuroendocrinology focuses on studies of central peptide function in three populations of subjects: (1) patients with major psychiatric illness, particularly affective illness, (2) patients with neuroendocrine disease, particularly Cushing's syndrome, and (3) normal controls. Some observations which have been made in these subjects of significance to biomedical research include: (1) identification of hypothalamic peptides with known central effects in experimental animals in the cerebrospinal fluid of normal human subjects and patients with major psychiatric illness, (2) elucidation of some aspects of the neuromodulation of peptide secretion into human CSF, (3) findings of abnormal patterns of peptide secretion into the CSF of patients with affective illness and anorexia nervosa and the determination of

significant relationships in peptide secretion into the CSF and plasma compartments, (4) demonstration of behavioral and cognitive effects of peptides in humans, and (5) development of a clinical neuroendocrine challenge paradigm which may be helpful in establishing the differential diagnosis between Cushing's disease and depression.

In our project to establish possible pathophysiological analogies between Cushing's disease and depression, we have taken a lead in applying techniques for studying human neurobiology in subjects with clinical neuroendocrine disorders. Since corticotropin releasing hormone may play an important role in the pathophysiology of both Cushing's disease and depression, we have also taken a lead in studies of its physiology in primate species. Thus, we were the first group to establish in primates a dose response curve for CRH effects on neuroendocrine function and to demonstrate its plasma half-life and metabolic clearance rate. We have also demonstrated that CRH seems to possess a profile of endocrine and physiologic effects which suggests that it is a major mediator of the stress response in primate species.

Proposed Course:

We shall continue active investigation into the secretion and regulation of several peptide hormones in human populations, particularly vasopressin, oxytocin, CRH, and related peptides (such as fragments of pro-opiomelanocortin). A new area of clinical study will focus on the behavioral, cognitive, and physiological responses to CRH administration in subjects with affective illness and Cushing's disease. We will test the hypotheses that a CRH challenge test may help in the diagnosis of depression and in the differential diagnosis of hypothalamic from pituitary Cushing's disease. A major new emphasis of the work in coming years will be in the laboratory. We have recently established radioimmunoassays for vasopressin, oxytocin, and ACTH, and are continuing on our work to validate our assay for CRH. These assays will not only be applied to human biological fluids but in extensive studies in sub-human primates utilizing intact animals, animals with pituitary stalk-sections, and animals with hypothalamic differentiation.

Publications:

Gold, P.W., Kaye, W., Robertson, G.L., and Ebert, M.: Abnormal regulation of arginine vasopressin in plasma and cerebrospinal fluid of patients with anorexia nervosa. New England Journal of Medicine, in press.

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Schulte, H.M., Chrousos, G.P., Gold, P.W., Oldfield, E.H., Phillips, J.M., Munson, P., Cutler, G.B., and Loriaux, D.L.: Metabolic clearance rate and plasma half-life of radioiodinated corticotropin releasing factor in a non-human primate. J. Clin. Endocrinol. Metab., in press.

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Becker, L.E., and Gold, P.W.: Analogies between Cushing's disease and depression. Hospital Psychiatry, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00449-08 CP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Outpatient Followup Studies of Manic-Depressive Patients and Families | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Yolande Davenport OTHER: Rex Cowdry Marvin L. Adland H. Arnold Meyersburg Sandra Bacon David Sack Leon Cytryn Donald McKnew Martine Lamour Marion Yarrow Carolyn Waxler Elliot Gershon | Chief, Unit on Family Studies Chief, Outpatient Unit Consultant Consultant Research Social Worker Staff Psychiatrist Medical Officer, (Research) Medical Officer, (Research) Medical Officer, (Research) Chief, Laboratory of Dev. Psy. Research Psychologist Chief, Unit on Psychogenetics | CP/NIMH CP/NIMH CP/NIMH CP/NIMH CP/NIMH CP/NIMH LDP/NIMH LDP/NIMH LDP/NIMH LDP/NIMH LCP/NIMH BP/NIMH |
| COOPERATING UNITS (if any) Unit on Psychogenetics, ABP/NIMH; Clinical Center Nursing Department. | | |
| LAB/BRANCH Clinical Psychobiology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.4 | PROFESSIONAL: 1.4 | OTHER: 1.4 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Research efforts of the Unit on Family Studies were focused this year in the following areas: collaborative studies of the cognitive and emotional development of children born to families where one parent has bipolar disorder; studies of early nurturing patterns of parents of infants at risk; follow-up and outcome studies of rapid-cycling bipolar patients; studies of psychosocial factors associated with seasonal affective disorders; a study of themes and therapeutic factors found in two groups of symptomatically distinct married bipolar patients; and a study of patients who left an NIMH psychobiology research ward AMA, and the impact of restructured staff roles on this phenomena. | | |

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

| | | |
|--------------------|------------------------------|-----------------|
| Catherine Connelly | Graduate Program Coordinator | G. Mason Univ. |
| John Nurnberger | Staff Fellow | BP/NIMH |
| Norman Rosenthal | Staff Psychiatrist | CP/NIMH |
| Laura Ryan | Nurse Clinician | CC Nursing Dept |
| Tina Gnagey | Staff Nurse | CC Nursing Dept |

Project Description

For the past ten years, the Outpatient Program of the CBP has been a collaborative project of the Staff of the Unit on Family Studies and the Outpatient Unit. In its early phases, the combined program focused on providing clinical care, (i.e., medication supervision and psychotherapy) on an outpatient basis to patients previously treated and studied on our inpatient ward. Accompanying our commitment to continuity of clinical care, a series of empirical studies were designed to examine psychocultural and psychodynamic features associated with bipolar patients and their families. Simultaneously, research was conducted regarding the efficacy of various psychotherapeutic approaches in the treatment and management of bipolar illness.

As a result of this work, an extensive series of articles was published describing the maladaptive character structure associated with the bipolar manic depressive personality, the interpersonal relationships of married bipolar patients and their spouses, and patterns noted to appear consistently in families where the disorder has occurred in more than one generation. Using a homogenous group psychotherapy modality, we outlined a comprehensive medical and psychodynamic approach to treatment. These "time-unlimited" psychotherapy groups, which consisted of bipolar patients, or bipolar patients with their spouses, also provided an excellent clinical resource both for the early identification and treatment of episodes, and for psychotherapeutic treatment of dysfunctional interactional patterns noted to persist between mood episodes which affect the quality of life for these patients and their families, in spite of medication.

Following an intensive evaluation, a decision was made to discontinue the long-term lithium maintenance and psychotherapy groups since the major clinical and research goals of the project had been accomplished. Patient care responsibilities were transferred during the early part of the year to the excellent private and clinic resources in the community. Several articles summarizing our long-term work with these patients are in preparation.

Termination of the long-term groups will permit a refocusing of research interests by both the Unit on Family Studies and the Outpatient Unit. It is anticipated that collaborative efforts of the two staffs will continue but to a lesser degree.

For example, for the past two years the Unit on Family Studies has sponsored and supervised the field placement of graduate social work students. The students are assigned to both the Inpatient Unit and Outpatient Clinic for their clinical experience. As a cooperative effort, the Family Studies Unit and the Outpatient Unit will continue to support a training program in psychological research for these students. Students will participate in a carefully supervised program of clinical assessment, psychotherapy and research. This training program is designed to illustrate the synthesis of the approaches of different disciplines, the ways in which clinical and biological research can synergize with rather than detract from clinical care, and an appreciation of the complex interactions between psychosocial factors and biologic predispositions.

Studies in process with other collaborators continue and will be elaborated upon in more detail in the following section.

Specific Projects

Although our project involving the treatment of married bipolar patients in conjunction with their spouses in group therapy ended this year, the efficacy of this modality has been described in previous papers. A summary of this work was published as a chapter in Major Psychopathology and Family Therapy. The therapeutic work with these patients appeared to interrupt the social isolation experienced by many bipolar patients and their families, and to prevent rehospitalization through early recognition of stress and by treatment of incipient episodes. These long-term groups provided a social continuity which seems important for these patients, as well as a setting in which faulty marital and family interactions could be explored and altered, and new patterns of response suggested.

Two subgroups of bipolar illness have been particularly well studied through the couples psychotherapy groups. One weekly group has focused on the male bipolar patient with "dysphoric" manias. The data accumulated from the three year course of this group are still to be analyzed. However, it is apparent that patients who experience dysphoric manias may have distinctive character structure and defensive styles persisting outside of the acute episodes, expressed through recurrent themes in group psychotherapy and interaction with spouses, parents, children, and authority figures in work settings.

A study of married rapid-cycling bipolar patients and their spouses who were seen over a nine month period in a couples' psychotherapy group is nearing completion. The index patients, previously hospitalized in the Inpatient Unit, presented stormy histories of rapidly alternating manic and depressive episodes, each phase lasting two to six weeks. On admission to the group, the rapid-cycling patients were relatively stable on medication. However, marital problems, some of which were necessarily dormant during earlier periods of coping with the illness and rearing children, surfaced and were brought to the psychotherapy group for discussion. The purpose of the study was to observe whether specific differences existed in the defensive techniques of families with a rapid-cycling member in contrast to bipolar families previously studied where episodes occurred less frequently, less predictably, and with long periods of euthymia between episodes. The following major patterns and themes have emerged: families may develop a phenomena we have termed "family cycling" in response to the cycling mood of the index patient; role diffusion is pronounced; fear of recurrence of the illness is more pervasive and anxiety producing in rapid-cycling families and the thematic degree of denial of affect, dependency, and loss is of the same magnitude found in other bipolar families. Although rapid-cycling illness is not rare, there is little in the literature describing the experience of these patients and their families, and the adjustments they make to the illness. The completed study will include a discussion of psychotherapeutic treatment of these families.

A study to examine history and outcome of patients previously hospitalized on the Inpatient Unit with a rapid-cycling mood disorder is under way. The

focus is on interim history since discharge from the Inpatient Unit, including the incidence of relapse and rehospitalization, the use of various treatment interventions, and the overall level of functioning achieved at the time of followup period. Preliminary data indicate that rapid-cyclers follow a highly variable course after discharge. However, at least three of these patients have independently discontinued all medications, with a marked improvement or cessation of their rapid-cycling affective disorder. Further results of our followup investigation will be reported in a summary article.

Although female bipolar patients are considered at special risk for recurrence of a child-birth related affective episode, there are substantially fewer studies of the reactions of men to becoming fathers, although men undergo emotional stress and their marital relationships are subject to significant change at the time of pregnancy. To test our hypothesis that episodes of manic-depressive disorder also occur in bipolar men in proximity to prospective fatherhood, a chart study was made of all former bipolar male patients admitted consecutively over a nine year period to our ward. Birth dates of all natural children and dates of hospitalization, as well as dates of outpatient episodes of mania and depression, were examined. The birth of a child was considered to have an influence on the occurrence of an episode when an episode occurred within nine months prior to or one year following the birth of the child. Family history of affective disorder, age of onset of affective illness in the patient, history of separation from the parent during the childhood years, and ordinal position in family of origin were among the parameters examined. Of the 40 male probands studied, it was found that 21 patients had a total of 25 episodes during the pregnancy of the spouse or within one year post-partum. This is in marked contrast to the rare occurrence of childbirth-related psychoses among males in the general population.

In contrast to reports of increased post-partum episodes found among women, the episodes found in the men tended to occur during the wife's pregnancy rather than following childbirth (16 pregnancy-related episodes compared to 9 post-partum episodes). More of the episodes were manic than depressed; 17 episodes required hospitalizations; eight episodes responded to outpatient treatment alone. The 21 men with birth-related episodes of illness tended to have a significantly earlier age of onset of manic-depressive disorder and a more severe illness than the 19 men without a pregnancy-related episode. These individuals also had a higher incidence of extended separation from parents during childhood. These data suggest that there may be a subgroup of male bipolar patients for whom fathering a child represents a sharply increased risk for perinatal or post-partum psychoses. Our findings strongly suggest the need for additional monitoring and ongoing psychotherapeutic support for both female and male bipolar patients during pregnancy and during the post-partum period. This additional attention may reduce stress, and it may enable early intervention to head off incipient affective episodes. Preventing affective episodes during this crucial period of a child's development may help insure the physical and emotional availability of both mother and father to facilitate the processes of attachment, individuation, and separation in the growing child. Findings from this study were published during the year.

Our collaborative research with the NIMH Laboratory of Developmental Psychology involving children born to parents treated in our groups continues. A symposium entitled "Study of Infant's of Parents with Bipolar Illness" in which all of the major collaborators appeared was held at the 1982 Annual Meeting of the American Psychiatric Association. Preliminary findings of the study were presented. When compared with their matched normal controls, children in bipolar families appear to be different according to investigators from the LDP, and blind raters from the University of Colorado. Children of bipolar parents tended to show extremes in their responses to the pain and sorrow of others and were either unusually empathic, or avoided the distress of others. During social interaction with peers, they displayed disruptive behavior in background climates of anger and conflict. When separated from their mothers, they showed more exploratory behavior than control children. They also showed more positive greeting behaviors but generally more avoidance upon the mothers return. In the home environment, the bipolar group displayed more frequent and severe behavioral problems than the normal control group. One of the instruments used in the study of the infant, the Ainsworth strange-separation paradigm provided the Unit on Family Studies with a design with which we could attempt to confirm in the reaction of parents to separation our earlier observations regarding the influence of antecedent patterns in perpetuating the disorder in the culture of bipolar families. Based on our understanding of the family psychodynamics, we had speculated that these patterns would manifest in the early stages of the nurturing experience of the new parents.

Our findings were based on the uses of several measurements including the Block Q-Sort which assessed differences on several parameters related to child-rearing. We found that few items dealing with issues of learning, achievement, and discipline-differentiated mothers from either normal or bipolar families. However, mothers from normal families appeared more attentive to their children's health needs than mothers in bipolar families. Both groups reported high values on achievement-related items, but mothers from bipolar families achieved a significantly higher rating (at $p < .10$) on one item they considered characteristic of their rearing philosophy, "I think it is a good practice for a child to perform in front of others." On childrearing items related to emotional issues, there were more significant contrasts. Mothers from bipolar families were less likely to encourage openness to experience in their children, which may have to do with the perfectionism found in bipolar families and fears of risk-taking. Mothers from bipolar families, more than normal control mothers also reported more negative affect toward their children; achieved lower ratings in the openness with which they expressed emotions in relation to their children; appeared to be more over-protective, and preferred their child not to try things if there was a chance the child might fail. Based on home visit observations, mothers from bipolar families were perceived as more disorganized and less active in interaction with their children. They also appeared more unhappy, tense, and ineffective. On a global functioning scale, when bipolar parents were rated at follow-up, scores were lowest in the areas of family interaction and social adjustment. The data presented in the symposium are in the process of preparation for an article.

A study measuring closeness preferences of adolescent offspring in schizophrenia neurotic and normal families was published this year. The study found

that normal families described attachments to the mother beyond those reported by schizophrenic and normal families, with the exception of schizophrenic patients who surpassed both next of age siblings and comparable control or normal offspring in closeness to mother. Within the families of a neurotic child, the intensities, splits, or coalitions observed affected all of the children in a somewhat similar pattern, even though one child in these families was more neurotically disturbed than others. An extension of this study, using families having an adolescent with bipolar illness and at least one "normal" adolescent sibling, is planned in order to illuminate family closeness patterns within the "bipolar family" and to compare the pattern with that observed in the "schizophrenic," "neurotic," and "normal" families previously studied.

A study of shifting interdisciplinary roles on an inpatient psychobiological research unit was completed. The data were presented at the 1981 Annual Meeting of the American Orthopsychiatric Association. Five years ago, the psychotherapy program on the ward was restructured to give much greater emphasis to the role of primary nurse clinicians and to de-emphasize the role of individual therapy and management by the research physician. Since this change, only one patient has been discharged from the ward against medical advice, in contrast to 19 patients discharged against medical advice in the previous 7 years. Paradoxically, this decrease in number of discharges against medical advice occurred at a time when the number and strenuousness of research procedures in which a patient might participate during hospitalization had increased markedly. A retrospective study of characteristics of the 20 AMA discharges emphasized the increasing involvement of the patient as an active collaborator in the research as a factor in minimizing discharges against medical advice. An article based on the study is in preparation.

Significance to biomedical research and to the program of the Institute:

Finally, the Unit on Family Studies is an active collaborator in two projects begun this past year. As part of the research design, psychosocial evaluations of patients with seasonal mood cycles, and patients who suffer from delayed sleep syndrome will be made. The Unit will provide individual, couples' and family psychotherapy on a limited basis and will refer to community resources when indicated.

Proposed course:

The long term research of the Unit remains focused on better understanding of the interplay between biogenetic factors and environmental events. The direction of the Unit will shift this year to more intensive study of sub-groups of patients with affective disorders and their families.

Publications:

Davenport, Y.B. and Adland, M.L.: Post-partum psychoses in female and male bipolar manic-depressive patients. Am. J. Orthopsychiatry 52:288-297, 1982.

Hoover, C. and Davenport, Y.B.: Family closeness of young schizophrenics: patterns of closeness in the families of schizophrenic, neurotic, and normal adolescents. Am. J. Family Therapy 9(4):52-60, 1981.

Connelly, C.E., Davenport, Y.B. and Nurnberger, J.I.: Adherence to treatment regimen in a lithium carbonate clinic. Arch. Gen. Psychiatry 39(5):585-588, 1982.

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|---|--|--|----------------|------------------------|---------|--------------------------|-------|---------|-------------|-------------------------------|---------|--------------|---------------|---------|---------------|--------------------------|---------------|-----------------|------------------------|---------------|------------|-----------------------|------------|-------------|-------------------------------|---------------|-----------------|---------------------------|---------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01852-04 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Brief Outpatient Studies of Depressive Syndromes and Character Disorders | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: Rex Cowdry</td> <td style="width: 40%;">Chief, Outpatient Unit</td> <td style="width: 30%;">CP/NIMH</td> </tr> <tr> <td>OTHER: Frederick Goodwin</td> <td>Chief</td> <td>CP/NIMH</td> </tr> <tr> <td>Thomas Wehr</td> <td>Chief, Clinical Research Unit</td> <td>CP/NIMH</td> </tr> <tr> <td>Sandra Bacon</td> <td>Social Worker</td> <td>CP/NIMH</td> </tr> <tr> <td>David Gardner</td> <td>Instructor in Psychiatry</td> <td>Georgetown U.</td> </tr> <tr> <td>Arthur Behrmann</td> <td>Resident in Psychiatry</td> <td>Georgetown U.</td> </tr> <tr> <td>Gay Grover</td> <td>Clinical Nurse Expert</td> <td>CC Nursing</td> </tr> <tr> <td>Thomas Wise</td> <td>Chairman, Dept. of Psychiatry</td> <td>Fairfax Hosp.</td> </tr> <tr> <td>David Goldstein</td> <td>Asst. Prof. of Psychiatry</td> <td>Georgetown U.</td> </tr> </table> | | | PI: Rex Cowdry | Chief, Outpatient Unit | CP/NIMH | OTHER: Frederick Goodwin | Chief | CP/NIMH | Thomas Wehr | Chief, Clinical Research Unit | CP/NIMH | Sandra Bacon | Social Worker | CP/NIMH | David Gardner | Instructor in Psychiatry | Georgetown U. | Arthur Behrmann | Resident in Psychiatry | Georgetown U. | Gay Grover | Clinical Nurse Expert | CC Nursing | Thomas Wise | Chairman, Dept. of Psychiatry | Fairfax Hosp. | David Goldstein | Asst. Prof. of Psychiatry | Georgetown U. |
| PI: Rex Cowdry | Chief, Outpatient Unit | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| OTHER: Frederick Goodwin | Chief | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thomas Wehr | Chief, Clinical Research Unit | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sandra Bacon | Social Worker | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| David Gardner | Instructor in Psychiatry | Georgetown U. | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arthur Behrmann | Resident in Psychiatry | Georgetown U. | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gay Grover | Clinical Nurse Expert | CC Nursing | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thomas Wise | Chairman, Dept. of Psychiatry | Fairfax Hosp. | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| David Goldstein | Asst. Prof. of Psychiatry | Georgetown U. | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS, (if any) Fairfax Hospital, Dept. of Psychiatry; Georgetown University, Department of Psychiatry. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Psychobiology Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2.2 | PROFESSIONAL: 2 | OTHER: 0.2 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Since the great majority of patients with <u>depressive syndromes and character disorders</u> are successfully treated in an outpatient setting, the Branch has placed a major emphasis on developing an outpatient facility for the clinical and biological evaluation and treatment of these patients. A comprehensive psychobiological evaluation of sixteen patients with depression spectrum illness has been performed, including circadian rhythm monitoring of temperature and activity, sleep electroencephalograms, TRH infusions, dexamethasone suppression tests, and collections of blood, urine, and cerebrospinal fluid. Global ratings of "endogenicity" correlate with <u>dexamethasone test</u> abnormalities and with early temperature minima. Post-dexamethasone cortisol correlates negatively with the rise in thyrotropin after <u>protirelin</u> . A study of the neurophysiology of <u>borderline syndromes</u> , including a multi-drug cross-over study, suggests that some symptoms in borderlines respond to carbamazepine. In addition, a small scale study of the effects of <u>tranylcypromine</u> on rapid-cycling bipolar illness suggests it may stop mood cycles in some patients. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

There has been increasing emphasis over the past several years in the study of the entire spectrum of depressive disorders, sparked by several findings. First, clinically homogeneous syndromes such as endogenous depression have been found to be biochemically heterogeneous, using tests such as the dexamethasone suppression test. Secondly, patients with non-endogenous depressions have been found to share some of the same biochemical abnormalities and drug responses as patients with more classical endogenous depressions. Third, specific behaviors, such as impulsive or suicidal behavior, which cut across diagnostic entities, have been associated with biological abnormalities such as low 5-hydroxyindoleacetic acid (5HIAA) in the spinal fluid. Because individuals with a wide spectrum of depressive disorders are commonly treated in outpatient settings, the outpatient program has developed a flexible and broad spectrum program for the evaluation of treatment of these disorders, requiring only brief inpatient stays. This approach helps assure a more representative sample of depressive disorders in our patient population, and reserves our inpatient resources for the longitudinal study of rapid-cycling and treatment-resistant patients.

The decision to cast a very broad diagnostic net for the biological studies allows for the study of clinical and biological dimensions crossing traditional diagnostic lines. Thus it is possible to examine whether impulsivity and suicidality are traits related to specific biological findings, across the depressive spectrum of illnesses. In addition, this approach allows for careful study of the interrelationship of biological markers. For example, simultaneous circadian temperature monitoring and recording of sleep EEG allow for conclusions about the possible association of phase-advance in the temperature rhythm with decreased REM latency, both findings having been reported as markers for endogenous depression. Similarly, possible association between dexamethasone non-suppression and a blunted response to TRH infusion can be examined in the population as a whole. This comprehensive approach also allows for replication of previous small scale studies associating cerebrospinal fluid metabolite findings with specific neuroendocrine findings, such as a blunted response to TRH. Such associations may give additional insight into possible hypothalamic regulatory mechanisms.

Sixteen patients were studied. Biochemical assays of cerebrospinal fluid, blood, and urine are incomplete. Global ratings of endogeneity within this group with major depressive illness are correlated with 1) the sporadic occurrence of temperature minima earlier than 3 AM and 2) post-dexamethasone cortisol levels. Post-dexamethasone cortisol levels did not identify drug responders; however, among dexamethasone-positive depressed patients, good responders showed a marked normalization of dexamethasone test results while non-responders did not normalize. Dexamethasone non-suppression, using 0.5 mg dexamethasone with an 8 AM cortisol level the next day, was strongly correlated with blunted thyrotropin (TSH) responses to protirelin (TRH).

The outpatient study of rapid-cycling bipolar affective disorder continued. In view of our previous findings of a very significant association between thyroid dysfunction and rapid-cycling illness, all patients entering into the

outpatient study received a thorough thyroid evaluation, usually including a TRH infusion. Twice daily ratings were performed on visual analog scales by the patient, and ratings of overall depression or mania were made by the patients' spouses and by therapists. Two of five rapid-cycling bipolar II patients treated with standard doses of tranylcypromine stopped cycling in a euthymic or mildly hypomanic state. The other three showed a lessening of intensity of the cycle, with less time spent in a depressed state. We have initiated clinical trials of suppressive dose of l-thyroxine (100-200 mcg, with suppression documented by protirelin infusions). Early results suggest that this approach changes the pattern of cycling, but clinical efficacy is unclear at this time.

The screening of possible participants in a long-term study of the borderline syndrome was begun this year. This study is based on a prior finding that individuals diagnosed as borderline have symptomatology suggestive of limbic system dysfunction and have a high incidence of abnormal, albeit largely non-specific, electroencephalograms. This study will examine electroencephalograms both in a routine clinical setting, under the stress of a semi-structured interview, and following procaine administration. In addition, the possibility of monoaminergic, particularly serotonergic dysfunction in these individuals with behavioral and affective dyscontrol will be examined through cerebrospinal fluid studies. A year-long double-blind placebo-controlled comparison of four medications suggested for use in the borderline syndrome (carbamazepine, alprazolam, tranylcypromine, and trifluoperazine) will follow the completion of the evaluation study. Five non-blind case studies and one blind treatment case suggest that carbamazepine may have dramatic effects on some components of the borderline syndrome, including perceptual distortions, acute dysphorias, and behavioral dyscontrol.

Significance to Biomedical Research and to the Program of the Institute:

Broad spectrum studies of depressive syndromes may help identify biological correlates of psychopathological dimensions which cut across traditional diagnostic boundaries, such as the association of low CSF 5-HIAA with behavioral dyscontrol. Demonstration of carbamazepine-responsive neurophysiological abnormalities in the borderline syndrome would radically alter our theories about this syndrome and provide a significant new treatment modality.

Proposed Course:

Data analysis of the depression-spectrum studies will be completed this year. The borderline studies will admit 15-20 patients during the next year for neurophysiological evaluation and treatment. This project number is discontinued. Results will be reported next year in the annual report of the Neurosciences Branch, IRP, NIMH.

Publications:

Cowdry, R.W. and Goodwin, F.K.: The dementia of bipolar illness: Diagnosis and response to lithium. Am. J. Psychiatry, 138:1118-1119, 1981.

Cowdry, R.W., Wehr, T.A., Zis, A.P. and Goodwin, F.K.: Thyroid abnormalities associated with rapid-cycling bipolar illness. Arch. Gen. Psychiatry, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01831-06 CP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Basic and Clinical Studies of Neuronal and Glial Enolase | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Paul J. Marangos OTHER: J.M. Polak A.G.E. Pearse S. Bloom D. Schmechel John Minna D. Carney A. Gazdar M. Brightman | Chief, Unit on Neurochemistry Lecturer Professor Lecturer Neurologist Chief, Medical Oncology Branch Oncologist Biochemist Chief, Sec. on Neurocytology | CP/NIMH Royal Med. Sch. London Royal Med. Sch. London Royal Med. Sch. London Duke Univ. NCI/NNMC NCI/NNMC NCI/NNMC LNNS/NINCDS |
| COOPERATING UNITS (if any) Royal Postgraduate Med. School, London; Duke Univ.: Laboratory of Neuro- path. and Neuroanatomical Sciences; NINCDS; Laboratory of Devel. Neurobiology, NICHD; Univ. of Connecticut; U. of Iowa; Oncol. Br., NCI; NNMC, Bethesda, MD. | | |
| LAB/BRANCH Clinical Psychobiology Branch | | |
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| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.7 | PROFESSIONAL: 0.6 | OTHER: 1.1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less, <u>underline keywords</u>) <u>Neuron specific enolase, NSE and non neuronal enolase, NNE</u> are specific bio- chemical markers for neurons and glia respectively. The NSE is also present in <u>neuroendocrine cells of the APUD classification</u> . Clinical studies with small cell lung cancer, neuroblastoma and pancreatic islet cell tumor patients has shown that serum NSE levels can be used to detect these conditions as well as monitor the clinical course of illness. Basic studies have shown that NSE appears during <u>development</u> at precisely the time when <u>synaptogenesis</u> is occurring. | | |

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

| | | |
|---------------|------------|-------------|
| Richard Prinz | Surgeon | Univ. Mich. |
| Ricardo Lloyd | Oncologist | Univ. Mich. |
| Paul Zeltzer | Oncologist | Univ. Texas |
| Robert Seeger | Oncologist | UCLA |

Project Description

Proteins are the functional molecules of the cell serving as enzymes, structural elements and receptors for various ligands. Genes are functionally expressed as specific protein molecules that either directly or indirectly control both the viability and identity of cells. The identification and characterization of proteins found only in specific cell types can therefore be expected to provide basic insights concerning the differentiated function of cells.

Our laboratory has been studying brain specific proteins in an effort to learn more about the basic biology of neuronal and glial function. During the last several years we have studied a protein formerly designated the 14-3-2 protein. We have purified the protein from brain and characterized it structurally functionally and immunologically. We have shown that this protein is a neuron specific form of the glycolytic enzyme enolase and named it neuron specific enolase (NSE). Extensive radioimmunological and immunocytochemical studies have shown that NSE is present in all neurons and that its appearance is correlated with neuronal differentiation. A second major form of enolase is also present in brain; we have purified this enzyme and shown it to be localized in glial cells. This form of enolase has been designated non-neuronal enolase or NNE. NNE and NSE are therefore unique in that they represent specific biochemical markers for the two major cell types in brain.

Several years ago we showed that NSE was also present in peptide secreting neuroendocrine cells in the periphery. In fact in the periphery the only cells which immunostain for NSE other than neurons are neuroendocrine cells of the amine precursor uptake and decarboxylation (APUD) classification. NSE has proven to be highly useful in this regard since we have shown in collaboration with Drs. Julia M. Polak and A.G.E. Pearse that virtually all APUD cells contain NSE. It is therefore possible to delineate the entire diffuse neuroendocrine system (DNES) of a given organ such as gut, lung or pancreas using antisera to NSE. We have shown that virtually all of the known regulatory peptide hormones, i.e., somatostatin, gastrin, VIP, CCK, etc., are present in cells which also contain NSE. In addition we routinely see cells of neuroendocrine appearance in the gut, lung and pancreas that do not stain for any known peptide indicating that these cells may contain peptides not yet isolated and characterized.

Our studies during the past year have focused on the clinical application of the NSE methodology. The high levels of NSE found in neuroendocrine tumors (APUDomas) have prompted us to examine the serum of these patients for elevated NSE levels. We have completed a study of 94 small cell lung cancer patients done in collaboration with Drs. Desmond Carney, John Minna and Adi Gazdar (NCI). In limited disease patients NSE was elevated in 40% of the cases whereas in extensive disease it was elevated in 87% of cases. More importantly the levels of NSE followed the clinical course of the illness, i.e., decreasing to normal levels during remission and returning to elevated levels upon relapse. NSE is proving to be one of the clearest biochemical markers available for small cell lung cancer. These clinical studies have been published in *Lancet*. We have also measured NSE levels in small cell cultures derived from patients. These cells, grown by Drs. Gazdar and Carney (NCI) have highly elevated levels of NSE in comparison to those cells obtained from non small cell lung cancer patients

indicating that the elevated serum NSE levels observed in small cell lung cancer patients is probably derived from these cells. These in vitro studies have been published in Cancer Letters.

Other APUDomas patients have also been examined and positive results were obtained in non functioning islet cell carcinoma. This work done in collaboration with Dr. Richard Prinz at Loyola University has shown that significant elevations in NSE serum levels are found in these patients and that the levels return to normal following surgery. These studies have been published in Lancet.

We have also studied in collaboration with Drs. Robert Seeger (UCLA) and Paul Zeltzer (Univ. of Texas, San Antonio) 122 stage IV neuroblastoma patients. Of these 117 had elevated NSE levels (96%). In the study of pediatric patients the NSE level was also correlated with the survival times indicating that not only is NSE a useful diagnostic agent for the neoplasm but that it can also provide information relating to illness severity. The results of these studies are being prepared for submission to Lancet. The NSE methodology has therefore proven useful in three different clinical situations, neuroblastoma, small cell lung cancer, and non-functioning islet cell carcinomas. In these diseases NSE serum levels should prove to be valuable in characterizing both disease extent as well as response to treatment.

In addition to the clinical studies we have also been actively pursuing basic studies involving NSE. The goal of these studies is to determine the physiological role of NSE, i.e., why has such a specific form of enolase evolved in the neuron. Developmental studies have shown that NSE only appears in the differentiated neuron with a genetic switch from NNE to NSE occurring at a late stage in neuronal development. The precise timing of the switch has been determined in both the chick central and peripheral nervous system. These studies done in collaboration with Drs. Whitehead and Maxwell at the University of Connecticut have shown that NSE appears in defined neuronal populations at a time that corresponds very closely to the process of these neurons establishing synaptic connections. These results establish that neuronal NSE content correlates with neuronal functions. These studies coupled with earlier reports that NSE is uniquely stable to chloride induced inactivation, indicate that NSE is probably present in the neuron in order to accommodate the unique ionic environment of this cell. The developmental studies mentioned have been published in Developmental Neuroscience and Developmental Brain Research.

The NSE methodology is beginning to find its place as an important tool in neurobiology. In collaboration with Polysciences we have now made the antiserum to NSE commercially available. Neuroanatomists, pathologists, neuroendocrinologists and oncologists have thus far shown great interest in NSE. The involvement of our group in this project has spanned the period of its matriculation from basic to clinical studies. Future studies will continue to focus on clinical application and investigations of other glycolytic enzymes in the brain.

Significance to biomedical research and to the program of the Institute:

NSE and NNE constitute a new methodology for studying the cellular structure of the brain and therefore relate directly to the program goals of the Institute. The enolase methodology is proving to be of great value to neuroanatomists, developmental biologists, neuropathologists, oncologists and neuroendocrinologists. Our recent focus on clinical applications has made clear the potential value of this system as a diagnostic tool in various neuroendocrine pathologies.

Proposed Course:

The interest generated in the NSE methodology by clinicians indicates that these studies will continue to develop within our laboratory over the next several years.

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Campbell, I.C., Marangos, P.J., Parma, A.M., Garrick, N.A. and Murphy, D.L.: Localization of monoamine oxidases A and B in primate brains relative to neuron specific and non-neuronal enolases. Neurochemical Research, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01833-02 CP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Adenosine Receptors in the Central Nervous Systems | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Paul J. Marangos OTHER: Jitendra Patel Jacqueline N. Crawley David Jacobowitz Jean-Phillipe Boulenger Jean-Claude Bisserbe Michael Lewis | Chief, Unit on Neurochemistry Fogarty International Fellow Research Scientist Chief, Sec. on Histopharmacology. Visiting Scientist Visiting Scientist Neuroanatomist | CP/NIMH CP/NIMH Dupont LCS/NIMH BPB/NIMH CP/NIMH MHRI/Univ. of MI. |
| COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH; Biological Psychiatry Branch, NIMH; Dupont Corporation; MHRI, University of Michigan. | | |
| LAB/BRANCH Clinical Psychobiology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.4 | PROFESSIONAL: 1.0 | OTHER: 0.4 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Summary of Work: Studies in our laboratory concerning the adenosine receptor system have expanded into several areas. We have described a specific binding site for [³ H] nitrobenzylthioinosine ([³ H] NBI) which probably represents the adenosine reuptake site. This site has a distinct pharmacology from that of the adenosine receptor. The interaction of caffeine with the adenosine and benzodiazepine receptor in human brain has been studied. The relationship of the calcium channel with adenosine receptors has also been studied. The binding of the calcium antagonist [³ H] nitrendipine, [³ H] NDP has been characterized with evidence generated showing that this compound is binding to the calcium channel. Behavioral studies have shown that the metabolically stable adenosine analogues cyclohexyladenosine and 2-chloroadenosine are extremely potent as sedatives and tranquilizers. Autoradiographic studies have also revealed that the adenosine receptor has a heterogeneous distribution in brain. The effect of Adenosine on ¹⁴ C 2-deoxyglucose uptake is also being studied in an attempt to correlate these metabolic effects with receptor distribution and to identify functional receptors. | | |

Project Description:

An important role for adenosine in neurotransmission has been suggested by many studies over the past decade, and the terms purinergic transmission and purinergic nerves have been coined to describe the role of this purine in neurotransmission. For example, adenosine has very potent effects on cAMP levels in brain; these effects are biphasic in nature depending on the purine concentrations employed and are thought to be mediated by an adenosine receptor. Adenosine also has been shown to inhibit presynaptic neurotransmitter release in a number of systems suggesting that it may play a role in the modulation of other neurotransmitter systems. A high affinity reuptake system has also been demonstrated for adenosine which seems to be distinct from the adenosine receptor since uptake inhibitors are poor inhibitors of the adenosine receptor.

Since adenosine has potent sedative effects and is an extremely potent inhibitor of neuronal firing it is likely that it plays a role in an important system in the brain that is involved in generalized inhibition of neuronal activity. It is therefore likely that the identification and characterization of specific adenosine receptors in brain will prove useful in understanding CNS depressant phenomena and also lead to the development of new psychotherapeutic agents. Direct study of [^3H] adenosine binding has proven to be difficult since this purine is rapidly broken down by the enzyme adenosine deaminase. In vitro membrane preparations also generate rather high levels of adenosine making binding studies difficult.

The recent availability of metabolically stable adenosine analogues has now made it possible to study adenosine receptors; these compounds bind to the receptor and are resistant to adenosine deaminase. Addition to membranes of adenosine deaminase destroys endogenously generated adenosine; the stable analogues N⁶ cyclohexyladenosine [^3H] CHA and diethylphenylxanthine, [^3H] DPX can then be used to easily demonstrate specific, saturable, high affinity adenosine receptors in brain membranes.

Studies in our laboratory over the past year have been relatively broad in scope. Dr. Patel, a Fogarty Visiting Fellow, has worked out and characterized [^3H] CHA binding in rat synaptosomal membranes. Two binding sites have been identified with respective K_D values of 0.7 and 2.4 nM. A detailed description of the binding site appears in last year's annual report. These rather extensive characterization studies have appeared in Brain Research. We have also performed autoradiographic studies and localized the receptor sites. High levels are found in the granule cell layer in cerebellar cortex, the hippocampus and the superficial layer of the superior colliculus. These studies done in collaboration with Dr. Michael Lewis at the University of Michigan have been published in the European Journal of Pharmacology. These preliminary studies will be continued in order to more completely describe the anatomical distribution of adenosine receptors and their relationship to alterations observed in ^{14}C -2-deoxyglucose uptake in response to adenosine receptor ligands.

Behavioral characterization of the metabolically stable ligands has been carried out in collaboration with Dr. Jacqueline N. Crawley (Dupont). Results

show that CHA and 2-chloroadenosine are extremely potent sedatives which effects at doses as low as 0.1 to 0.5 mg/kg. These studies appeared in Life Sciences and will be extended using adenosine antagonists such as caffeine in an attempt to block adenosine receptor mediated behaviors.

In collaboration with Dr. Jean-Phillip Boulenger (BPB) we have compared the inhibitory potency of caffeine on the adenosine and benzodiazepine receptor in human brain membranes. These studies which have been published in Neuroscience Letters have shown that caffeine is about 10 fold more potent as an inhibitor of adenosine receptors than benzodiazepine receptors. In both systems caffeine was shown to competitively inhibit binding. In an effort to determine which receptor system caffeine preferentially interacts with *in vivo* we administered caffeine chronically to mice and assessed the tone of both adenosine and benzodiazepine receptors at 12, 26, and 40 days. Two doses of caffeine were used one giving a dose of 50 mg/kg/day and the other 100 mg/kg/day. Brain benzodiazepine receptors were elevated (increase in receptor number) at day 12 in the high caffeine dosed animals while no change was observed at the other dose and time points. Adenosine receptors were significantly increased at all time points with kinetic analysis showing that the increased binding was due to an increased number of receptors. It therefore appears that chronic caffeine administration affects both adenosine and benzodiazepine receptors with the former being affected to a greater degree. These studies suggest that these two receptor systems are up-regulated in response to caffeine and provides further evidence that caffeine is an antagonist in both systems.

We have also studied the adenosine uptake site in brain during the past year. Using [^3H] nitrobenzylthioinosine, [^3H] NBI, a potent adenosine uptake blocker, we have shown that distinct adenosine uptake binding sites exist in a number of tissues including brain. The binding of [^3H] NBI is saturable, specific and has a high affinity ($K_D = 0.1 \text{ nM}$). Adenosine receptor ligands such as CHA and 2-chloroadenosine are very poor inhibitors of [^3H] NBI binding indicating that the adenosine uptake site is distinct from that of the adenosine receptor. This is the first demonstration of specific binding to the adenosine uptake site in brain and will be reported in the July issue of Journal of Neurochemistry. Pharmacologic manipulation of the adenosine uptake site may prove to have behavioral consequences. For example, in collaboration with Dr. Crawley we have shown that pretreatment of mice with NBI lowers the dose of adenosine required to produce behavioral sedation. This effect is probably due to the presence of increased levels of adenosine at the synapse following reuptake inhibition. In an additional series of studies we have shown that the benzodiazepines are poor inhibitors of [^3H] NBI binding, suggesting that these drugs are not, at therapeutic levels, affecting the adenosine uptake site. These studies have been published in Neuroscience Letters.

Adenosine is known to modulate neurotransmitter release, a process that is dependent on calcium. This observation coupled with our finding that calcium effects the binding of [^3H] CHA to the adenosine receptor prompted us to examine the effects of the calcium antagonist nifedipine on [^3H] CHA binding. Nifedipine was shown to be a potent competitive inhibitor of [^3H] CHA binding suggesting that the calcium ionophore may be coupled to adenosine receptor. We have therefore, initiated a study of [^3H] nitrendipine binding in brain. A

highly specific, saturable, high affinity binding site has been demonstrated in brain membranes that satisfies many of the requirements for being considered a calcium channel. Binding is inhibited by EDTA and EGTA as well as other calcium antagonists such as verapamil. Incorporation of calcium into calcium free membranes nearly doubles the number of observed binding sites. The binding site appears to be a protein since preincubation of membranes with low levels of proteolytic enzymes or sulphydryl reagents such as iodoacetamide and dithiothreitol decreases specific binding by over 90%. These studies will be published in Life Sciences. Further characterization of the calcium channel in brain should provide insights into excitation-secretion processes in nervous tissue and may lead to the development of new psychotherapeutic agents. Studies presently in progress will determine whether various calcium antagonists can penetrate the blood-brain barrier. Behavioral studies will compare the effects of both intraperitoneal and intracerebroventricular injection of nifedipine and verapamil. During the past 4 months, largely due to the efforts of Dr. Jean-Claude Bisserbe, a visiting scientist from France, we have set up the 2-deoxyglucose method in our laboratory. We hope to localize functional adenosine receptors using this method. Regional changes in 2-deoxyglucose uptake in response to various adenosine receptor ligands will be correlated with our receptor autoradiography patterns. These data should provide important information about the identity and location of specific functional receptor populations.

Further studies of the adenosine receptor, the adenosine uptake site and the calcium channel are expected to provide new insights concerning the modulation of neuronal activity and also lead to the development of new pharmacologic agents.

Significance to Biomedical Research and to the Program of the Institute:

Determining the functions of the newly discovered neuromodulator system in brain is consistent with the program goals of the Institute in that it will increase our knowledge of the biochemical basis of behavior. Adenosine obviously mediates the functional tone of many brain neurons and the ability to pharmacologically intervene in this system will likely prove to be of clinical relevance.

Proposed Course:

These studies are expected to continue over the course of the next several years.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01834-05 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Endogenous Ligands for the Brain Benzodiazepine Receptor | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: Paul J. Marangos</td> <td style="width: 40%;">Chief, Unit on Neurochemistry</td> <td style="width: 30%;">CP/NIMH</td> </tr> <tr> <td>OTHER: Jitendra Patel</td> <td>Fogarty Fellow</td> <td>CP/NIMH</td> </tr> <tr> <td>Jean-Claude Bisserbe</td> <td>Visiting Scientist</td> <td>CP/NIMH</td> </tr> <tr> <td>Phil Skolnick</td> <td>Pharmacologist</td> <td>LBC/NIAMDD</td> </tr> <tr> <td>Jean Phillip Boulenger</td> <td>Visiting Scientist</td> <td>BPB/NIMH</td> </tr> <tr> <td>Jacqueline Crawley</td> <td>Research Scientist</td> <td>Dupont</td> </tr> <tr> <td>Steven M. Paul</td> <td>Chief, Unit on Preclinical Pharm.</td> <td>CP/NIMH</td> </tr> <tr> <td>Anna Wirz-Justice</td> <td>Biochemist</td> <td>Psych. Univ. Klinik, Switzerland</td> </tr> <tr> <td>Marion Kafka</td> <td>Pharmacologist</td> <td>BPB/NIMH</td> </tr> </table> | | | PI: Paul J. Marangos | Chief, Unit on Neurochemistry | CP/NIMH | OTHER: Jitendra Patel | Fogarty Fellow | CP/NIMH | Jean-Claude Bisserbe | Visiting Scientist | CP/NIMH | Phil Skolnick | Pharmacologist | LBC/NIAMDD | Jean Phillip Boulenger | Visiting Scientist | BPB/NIMH | Jacqueline Crawley | Research Scientist | Dupont | Steven M. Paul | Chief, Unit on Preclinical Pharm. | CP/NIMH | Anna Wirz-Justice | Biochemist | Psych. Univ. Klinik, Switzerland | Marion Kafka | Pharmacologist | BPB/NIMH |
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| Marion Kafka | Pharmacologist | BPB/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIAMDD; Biological Psychiatry Branch, NIMH; E.I. Dupont Company, Glenolden, Pennsylvania; Psychiatrische Universitäts Klinik, Basel, Switzerland. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Psychobiology Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.4 | PROFESSIONAL: 0.8 | OTHER: 0.6 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <p> The <u>benzodiazepines</u> probably exert their actions via interaction with specific <u>receptor sites</u> on neurons. Studies during the last year have focused on the <u>characterization of the benzodiazepine receptor</u> using newly available ligands. Studies involving the <u>β-carboline-3-carboxylate ethyl ester (β-CCE)</u> showed that the binding of both this ligand and the <u>peripheral type receptor ligand</u>, <u>RO-5-4864</u>, are not affected by GABA. Specific <u>peripheral type benzodiazepine</u> <u>receptors</u> were described in brain and characterized. These studies have shown that <u>benzodiazepine agonists</u> and <u>antagonists</u> interact differently with this receptor. The interaction of the <u>methylxanthine stimulant</u>, <u>caffeine</u>, with the benzodiazepine receptor in human brain has been studied; its relative potencies in binding to the <u>adenosine receptor</u> and the benzodiazepine receptor were compared. The finding that <u>caffeine</u> is more potent as an inhibitor of adenosine receptor binding suggests that the <u>adenosine receptor system</u> may mediate some of the <u>anxiogenic effects</u> of this <u>cortical stimulant</u>. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description

The development of psychoactive drugs over the past several decades has largely taken place by a process of trial and error. Compounds that can traverse the blood-brain barrier have been synthesized and subjected to behavioral analysis. Those possessing behavioral properties, i.e., stimulants, sedatives, tranquilizers, antipsychotics, have generally found a role as therapeutic agents. With the increased basic understanding of neurotransmission and neuromodulation it is now becoming feasible to design drugs that effect specific systems in brain. In some cases it is also possible to use behaviorally characterized drugs as probes to increase our understanding of the biological substrates of various behavioral phenomena.

The benzodiazepines are behaviorally quite well characterized and have been shown to be effective anxiolytics, anticonvulsants, muscle relaxants, and at higher doses, sedative-hypnotics. The discovery in 1977 that [^3H] diazepam binds to specific receptors in the brain suggested a number of interesting possibilities concerning the use of these drugs as specific probes for the study of systems in brain that might mediate anxiety. Some of the more obvious questions raised by the demonstration of specific receptors for the benzodiazepines were: are there specific endogenous ligands for this receptor and do these agents function to mediate levels of excitability in various defined areas of brain; what is the physiology of this receptor and how does it effect neuronal excitability; and, finally, is this receptor system altered in various psychiatric or neurologic disease states?

Our studies concerning the benzodiazepine receptor and determination of its putative endogenous ligand have focused on several areas during the past year. We have studied the interaction of various benzodiazepines and β -carbolines with the receptor. Specifically, using the newly available peripheral type benzodiazepine receptor ligand [^3H] RO-5-4864, we have shown that peripheral type receptors exist in brain. This ligand specifically binds to the kidney type benzodiazepine receptor. We found a peripheral type receptor in brain and have characterized its binding. There are about 25% as many "peripheral" type receptors in brain as there are central type sites. The binding site is saturable, high affinity ($K_D = 1\text{nM}$) and present at high levels in the crude nuclear fraction. One of the most interesting findings of this study is that binding of [^3H] RO-5-4864 is not affected by GABA. Micromolar levels of GABA increase the affinity of [^3H] diazepam for the central type receptor by two-fold whereas absolutely no effect is observed on brain binding of [^3H] RO-5-4864 at millimolar levels of GABA.

This result suggests that the peripheral type benzodiazepine receptor is probably not coupled to the GABA receptor-chloride ionophore complex as the central type receptor has been suggested to be. The function of the benzodiazepine binding site in brain is not known and is presently under study. These studies will be published in Neuroscience Letters and Molecular Pharmacology.

Follow-up studies have also been performed on β -carboline binding to the benzodiazepine receptor. Here we have used [^3H] β -carboline-3-carboxylate ethyl ester (β -CCE). We have also shown that GABA has no effect on the binding

of [^3H] β -CCE to brain membranes. Since the β -carbolines have behavioral actions opposite to those of the benzodiazepines we have postulated that agonists and antagonists interact in a different manner with the benzodiazepine receptor. It is likely that different conformations may be induced by agonists (β -carbolines) than antagonists (benzodiazepines) and that only the latter conformation is responsive to GABA. These studies have appeared in Life Sciences.

During the past four months we have been developing a methodology for studying calcium-calmodulin stimulated protein phosphorylation in brain synaptosomal membranes. This system utilizes ^{32}P -ATP as a substrate and the proteins are then separated on a polyacrylamide slab gel. The gel is fixed, dried, and then placed on a photographic film where an autoradiographic print is produced. The phosphorylated proteins appear as bands and their amount can be measured using a densitometer. We are attempting to use this system to determine whether the benzodiazepines effect the phosphorylation of brain proteins and whether these effects can be related to receptor binding potencies. Our preliminary results in this area indicate that the benzodiazepines inhibit calmodulin-induced phosphorylation in a manner which suggests that these drugs may be interacting directly with calmodulin. Studies are currently in progress aimed at determining whether this rather marked effect can be reversed by benzodiazepine antagonists. Studies are also being planned where benzodiazepines will be injected into animals and various brain regions will be examined for changes in protein phosphorylation patterns.

These new protein phosphorylation studies are rather complex and time consuming; we hope to apply them in several different areas. Protein phosphorylation is probably one of the major mechanisms of macromolecular modulation in brain and its characterization will doubtless provide important insights into brain function. Preliminary results have shown that several benzodiazepines inhibit calmodulin-induced protein phosphorylation. The inhibition seems to be general in nature with many proteins being affected. Further studies are in progress to determine the effects of various benzodiazepine antagonists on this inhibition of protein phosphorylation by diazepam.

Dr. Jean-Claude Bisserbe, a guest Visiting Scientist from France, has also during the last 4 months worked out in our laboratory the procedures involved in the 2-deoxyglucose method. We plan to determine whether ligands for the benzodiazepine and adenosine receptor induce changes in 2-deoxyglucose uptake in brain that correlate with the receptor distribution. We hope this approach will enable us to identify functional receptor sites.

Significance to Biomedical Research and to the Program of the Institute:

The studies described are expected to yield a clearer understanding of the biomedical mechanisms underlying anxiety and seizure generation. These phenomena are of interest in psychiatric disorders where anxiety is a major component, and in epilepsy. It is also expected that the protein phosphorylation and 2-deoxyglucose methodologies will significantly expand our understanding of the ways in which benzodiazepines and other psychoactive drugs affect brain function.

Proposed Course:

The studies described will shift in emphasis from receptor binding assays to functional correlates of receptor binding. Attempts will be made to show regional metabolic and macromolecular alterations in response to drug treatment. These functional receptor studies are likely to continue for several years.

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Marangos, P.J., and Crawley, J.N.: Chronic benzodiazepine treatment increases ³H muscimol binding in mouse brain. Neuropharmacology, 21:81-84, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01836-04 CP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Receptors for Neurotransmitters, Neuropeptides, and Neuromodulators in the C.N.S. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">P.I.:</td> <td style="width: 40%;">Steven M. Paul</td> <td style="width: 30%;">Chief, Clinical Neuroscience Branch</td> <td style="width: 10%;">CP/NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Phil Skolnick</td> <td>Pharmacologist</td> <td>LBC/NIADDK</td> </tr> <tr> <td></td> <td>Frederick Goodwin</td> <td>Director</td> <td>IRP/NIMH</td> </tr> <tr> <td></td> <td>Paul Marangos</td> <td>Chief, Unit on Neurochemistry</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Moshe Rehavi</td> <td>Visiting Fellow</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Sally Hays</td> <td>Visiting Scientist</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Richard Hauger</td> <td>PRAT Fellow</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Jacqueline Crawley</td> <td>Research Associate</td> <td>CP/NIMH</td> </tr> <tr> <td></td> <td>Jeffrey Barker</td> <td>Medical Officer</td> <td>LNP/NINCDS</td> </tr> <tr> <td></td> <td>Michael Brownstein</td> <td>Medical Officer</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Kenner Rice</td> <td>Pharmacologist</td> <td>LC/NIADDK</td> </tr> <tr> <td></td> <td>Wallace Mendelson</td> <td>Staff Psychiatrist</td> <td>BPB/NIMH</td> </tr> <tr> <td></td> <td>Ellis Kempner</td> <td>Physician</td> <td>LPB/NIADDK</td> </tr> </table> | | | P.I.: | Steven M. Paul | Chief, Clinical Neuroscience Branch | CP/NIMH | OTHER: | Phil Skolnick | Pharmacologist | LBC/NIADDK | | Frederick Goodwin | Director | IRP/NIMH | | Paul Marangos | Chief, Unit on Neurochemistry | CP/NIMH | | Moshe Rehavi | Visiting Fellow | CP/NIMH | | Sally Hays | Visiting Scientist | CP/NIMH | | Richard Hauger | PRAT Fellow | CP/NIMH | | Jacqueline Crawley | Research Associate | CP/NIMH | | Jeffrey Barker | Medical Officer | LNP/NINCDS | | Michael Brownstein | Medical Officer | LCS/NIMH | | Kenner Rice | Pharmacologist | LC/NIADDK | | Wallace Mendelson | Staff Psychiatrist | BPB/NIMH | | Ellis Kempner | Physician | LPB/NIADDK |
| P.I.: | Steven M. Paul | Chief, Clinical Neuroscience Branch | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | Frederick Goodwin | Director | IRP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Paul Marangos | Chief, Unit on Neurochemistry | CP/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIADDK; Laboratory of Chemistry, NIADDK; Biological Psychiatry Branch, NIMH; Laboratory of Physical Biology, NIADDK | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Clinical Psychobiology Branch | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION National Institute of Mental Health, IRP, Bethesda, MD 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) High affinity, stereospecific recognition sites (<u>receptors</u>) for putative neurotransmitters, neuromodulators, and many psychotherapeutic agents have been identified in the mammalian <u>central nervous system</u> . It is currently believed that the interaction of a neurotransmitter, neuromodulator, or psychotherapeutic agent with these sites initiates a series of events resulting in either a physiologic/behavioral response (in the case of neurotransmitters and neuromodulators) or a pharmacologic action (in the case of a psychotherapeutic agent). Furthermore, the high affinity, stereoselective binding of psychotherapeutic agents to brain also suggests that previously undescribed <u>endogenous modulators</u> , which physiologically mimic (or antagonize) the actions of these agents may be present in brain. Several receptor systems are currently under study, including: a) <u>benzodiazepine receptors</u> (believed to play a role in the pathophysiology of anxiety, seizure disorders, <u>musculoskeletal disorders</u> , and <u>sleep disorders</u>); b) <u>adenosine receptors</u> (believed to be involved in <u>sleep/wakefulness</u>); c) receptors for <u>tricyclic antidepressants</u> ; d) receptors for <u>central stimulants</u> (e.g., amphetamine); e) receptors for <u>hallucinogens</u> (e.g. <u>phencyclidine</u>). | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: To characterize and determine the physiological, pathophysiological, and pharmacologic role(s) of receptors for neurotransmitters, neuromodulators, and psychotherapeutic agents in the central nervous system. To isolate, identify, and characterize endogenous modulators (ligands) of these receptors. To examine the effects of behavioral, physiologic, and pharmacologic intervention on the plasticity of these recognition sites. To determine structure-activity relationships of chemically modified substances related to putative endogenous ligands and/or psychotherapeutic agents. To design and develop potential therapeutic agents and neurochemical tools with the information derived from the above studies.

Methods Employed: Radioreceptor techniques have been employed in the neurochemical characterization of receptor sites and as a means of detecting and quantitating endogenous substances which may regulate these sites. Both brain and peripheral tissues have been employed in these studies, the latter primarily for use as a biological "marker" in human studies where obtaining brain tissue would be impractical. Radioenzymatic techniques combined with thin layer chromatography or ion-exchange chromatography have been used to examine the role of adenosine deaminase in the sedative/hypnotic actions of adenosine and to obtain the target size of brain acetylcholinesterase by radiation inactivation. Other biochemical techniques employed include: high pressure liquid chromatography, gel filtration, molecular size exclusion chromatography. Pharmacologic testing has been accomplished by quantitation of the sensitivity of mice to the chemical convulsant, pentylenetetrazole (PTZ); monitoring ataxia and muscle relaxation through the use of a rotating rod and "wire-grip" procedures; anxiolytic action using either a mouse model of behavior (which measures the activity of mice in a novel environment), or alternatively, using a rat "conflict" model of behavior (the thirsty rat conflict test). Blood pressure in rodents has been measured using an indirect (tail cuff) technique; anxiolytic/anxiogenic behavior in primates is currently being investigated using behavioral rating scales (modified Redman) as well as using somatic markers of anxiety (pulse rate, mean arterial blood pressure, and plasma glucocorticoids determined by radioimmunoassay).

Major Findings: Previous studies from this laboratory have demonstrated that recognition sites for benzodiazepines are functionally linked to receptors for the major inhibitory neurotransmitter, GABA, and a chloride ionophore. The functional interaction of these three sites as part of a "supramolecular complex" has been useful in explaining the actions of compounds such as barbiturates and pyrazolopyridines, which share common pharmacologic actions with benzodiazepines but do not bind to benzodiazepine recognition sites. We have also previously demonstrated that certain C-3 substituted β -carbolines, which also bind to benzodiazepine receptors with high affinity, antagonize both the anxiolytic and anticonvulsant actions of benzodiazepines such as diazepam. The availability of tritiated, C-3 substituted β -carbolines with high specific radioactivity permitted us to compare the regulatory properties of benzodiazepine receptors when occupied by functional "agonists" and "antagonists".

In contrast to the apparent increased affinity of such receptors for agonist benzodiazepines observed in the presence of GABA, barbiturates, pyrazolopyridines, or chloride ions there is no increase in apparent affinity for the benzodiazepine antagonist 3-carboethoxy- β -carboline (β -CCE). This finding was extended to another structurally unrelated benzodiazepine antagonist, CGS-8216 (a pyrazoloquinolinone), suggesting the lack of regulation of benzodiazepine receptor affinity in the presence of modulatory agents (such as GABA) may be a common property of a variety of benzodiazepine receptor antagonists.

These observations have led to the development of a rapid, sensitive in vitro test which differentiates benzodiazepine-like compounds from antagonists. This screening procedure has proven to have excellent predictive value by post hoc analysis.

Studies to determine the molecular target size by radiation inactivation of benzodiazepine receptors when occupied by radiolabelled agonists and antagonists has shown the target size to be virtually identical, approximately 60,000 daltons.

Fundamental differences in the regulatory properties of the benzodiazepine receptor complex when occupied by an agonist or antagonist also prompted studies using an irreversible benzodiazepine, irazepine. Incubation of cerebellar membranes with irazepine followed by ligand binding studies using either [^3H] diazepam or β -CCE resulted in a loss of [^3H] diazepam binding sites (with a small accompanying change in apparent affinity) while an affinity change (with little or no change in site number) was observed using [^3H] β -CCE as a radioligand. This observation, taken together with molecular target size and kinetic studies, suggests that benzodiazepine agonists and antagonists bind to separate subsites or "domains" on the benzodiazepine receptor. The presence of domains on a protein with a molecular size of approximately 60,000 daltons (the "benzodiazepine receptor") for agonists and antagonists would explain the lack of enhancement of apparent receptor affinity by (e.g.) GABA and barbiturates in the presence of an antagonist, and is also consonant with kinetic studies suggesting agonists and antagonists bind to the same macromolecular entity. Similar results have recently been observed using a chemically unrelated benzodiazepine antagonist, RO 15-1788, further supporting the domain concept.

The most extensively studied β -carbolines with benzodiazepine antagonist properties, β -CCE and 3-hydroxymethyl- β -carboline (3-HMC), do not appear to have intrinsic convulsant activity. However, the methyl analog of β -CCE (3-carbo-methoxy- β -carboline, β -CCM) has recently been demonstrated to produce motor seizures in mice. These seizures could be effectively antagonized by diazepam, as well as the benzodiazepine antagonists RO-15-1788 and CGS-8216, suggesting that occupation of either an agonist or antagonist domain may be sufficient to interrupt or prevent these seizures. Marked differences in susceptibility to the convulsant actions have been observed between strains of mice. The molecular bases of this strain difference is currently being investigated.

3-HMC has been previously demonstrated to antagonize the anxiolytic and anticonvulsant actions of diazepam. We have used this compound as a tool to

investigate the role of the benzodiazepine receptor in the sedative/hypnotic actions of benzodiazepines. Administration of 3-HMC at doses (7.5 mg/kg, i.p.) which do not affect basal sleep parameters (defined electroencephalographically) in rats significantly antagonized the hypnotic actions of flurazepam, reflected in a significant reversal of flurazepam-elicited decreases in sleep latency and increases in total sleep time. Furthermore, slightly higher doses of 3-HMC elicited a significant increase in sleep latency and decreased total sleep time over a two hour recording period. These observations are the first definitive demonstration that the benzodiazepine receptor mediates the hypnotic actions of the benzodiazepines, and also strongly suggests the benzodiazepine receptor is involved in the physiologic control of sleep. These alterations in sleep patterns of the rat were not accompanied by significant changes in motor activity. In contrast to analeptics such as caffeine and amphetamine which elicit similar changes in EEG defined sleep but invariably also cause large increases in motor activity, 3-HMC appears to possess only the somnolytic action. These observations suggest that 3-HMC or a related compound could be potentially useful as a therapeutic agent in disease states associated with excess sleepiness.

Pilot studies are now in progress to examine whether some benzodiazepine antagonists (such as β -CCE) have anxiety producing actions (anxiogenic) in primates. Preliminary results have shown administration of β -CCE to be associated with rapid, marked elevations in plasma cortisol, as well as dramatic increases in mean arterial blood pressure and pulse rate. Pretreatment of monkeys with diazepam or RO 15-1788 effectively antagonized these somatic manifestations of anxiety.

Previous studies have shown that administration of a reversible inhibitor of adenosine deaminase (EHNA; 9-(erythrohydroxynonyl adenine) results in a profound reduction in motor activity (a behavioral sedation) which is temporally paralleled by an inhibition of brain adenosine deaminase activity. However, electroencephalographic measurement of these "behaviorally sedated" rates demonstrated significant reductions in total sleep time and dramatic increases in sleep latency. This paradoxical behavior has been termed "quiescent waking".

Administration of L-phenylisopropyladenosine (L-PIA), a potent analog of adenosine (which binds to adenosine A_1 receptors with an affinity of less than 10 nM) resulted in a long lasting behavioral sedation. However, EEG changes appear similar to those produced by EHNA. These observations suggest that although adenosine is behaviorally sedating, the electroencephalographic patterns are reminiscent of a behavioral activation. It had been proposed that the sedation elicited by adenosine and adenosine derivatives may be due to the hypotensive actions of these compounds. Indeed, administration of as little as 0.1 mg/kg of L-PIA elicits a profound reduction in the blood pressure of anaesthetized rats. However, administration of 8-p-sulfophenyltheophylline, an adenosine antagonist which does not cross the blood-brain barrier, resulted in a significant reversal of the hypotension produced by L-PIA, but does not reverse the behavioral sedation. These observations provide strong evidence that the sedation produced by L-PIA (and EHNA) is centrally rather than peripherally mediated.

Neurochemical studies suggest that a circadian rhythm in the number of adenosine A₁-receptors occur in rat hippocampus and cortex, but not cerebellum. Studies are currently in progress to further investigate the physiological significance of these changes with regard to altered sleep patterns in rats.

Previous studies have demonstrated that [³H] imipramine labels a "serotonin transporter" (recognition site + transport protein) in brain and platelet of both human and rat. The number of these sites is profoundly reduced in platelets of depressed patients, yet does not return to "normal" values following clinical improvement of depression, suggesting this parameter could be an important marker for depressive illness. The demonstration of a strong concordance of [³H] imipramine binding to platelets from monozygotic (identical) twins further strengthens the contention that this parameter may be a useful "biological marker" in depression. Studies are now in progress to solubilize the serotonin transporter and separate a recognition site for serotonin (and tricyclic antidepressants) from a transport protein, with the ultimate goal of reconstitution of this system in artificial membranes.

High affinity, saturable and stereospecific recognition sites for amphetamine (and related phenethylamines) has been demonstrated in mammalian brain. The highest densities of these sites are in hypothalamus and striatum, yet pharmacologic characterization of these sites suggest it is not related to recognition sites for any previously described neurotransmitter. Furthermore, a highly significant ($p < 0.01$; $r = 0.97$) correlation has been demonstrated between the potencies of a series of phenethylamine derivatives to displace [³H] amphetamine from hypothalamic membranes and their potencies as anorectic agents. A similar correlation was not obtained for the motor stimulant properties of these compounds, suggesting these sites may be a locus of the appetite suppressing properties of amphetamine and related compounds.

A stereospecific inhibition of [³H] phencyclidine (³H-PCP) binding to brain slices has been observed using an enantiomeric pair of PCP analogs (phenylcyclohexyl-3-methylpiperidine; PCMP). The differences in potency observed for this enantiomeric pair is in good agreement with the behavioral and electrophysiological differences already reported by this laboratory.

Previous studies in our laboratory demonstrated the binding of biologically active ¹²⁵I] cholecystokinin (CCK) to rat brain membranes. This binding was of high affinity, saturable, reversible and inhibited by biologically-active cholecystokinin peptides. Kinetic and competition studies suggest that these high affinity binding sites represent physiologically relevant receptors for CCK in brain. Characterization of the cellular elements in which CCK receptors are concentrated in the basal ganglia was carried out by a series of chemical and physical lesion studies. These experiments revealed that CCK receptors are highly localized to intrinsic neuronal elements in the caudate nucleus with at least 75% of the receptors on neuronal cell bodies. Postmortem studies of the brain areas from patients with Huntington's chorea confirmed the marked localization of CCK receptors to neurons of the basal ganglia since a marked decrease was observed in the brains of Huntington's patients when compared to age-matched controls. More recent studies have also demonstrated marked decreases in the density of CCK receptors in affected areas of cerebral cortex

in patients dying of Huntington's disease. The latter findings suggest that CCK receptors may be involved in higher cortical (cognitive) functions.

Studies on the binding of [^3H] ouabain to brain Na-K ATPase have also been conducted. [^3H] Ouabain binds to two distinct "high affinity" sites in various areas of rat brain; whereas human brain and erythrocyte ghosts only have one high affinity site. In the rat striatum "high affinity" 17.4 and 400 nM and binding capacities of 12.0 and 36.0 pmol/mg, respectively. Human erythrocyte ouabain receptors exhibited an apparent K_D of 9.6 nM and binding capacity of 0.94 pmol/mg. In contrast "high affinity" sites were absent from peripheral tissues i.e. heart and kidney. Following lesions of the rat striatum with kainic acid, a marked decrease (40-60%) of both "high affinity" ouabain binding sites was neuronal $\text{Na}^+\text{-K}^+$ ATPase. This hypothesis was further supported by studies on postmortem brain samples from patients with Huntington's disease where a marked decrease in [^3H] ouabain binding was observed in the caudate nucleus when compared to matched controls. Current studies are directed toward understanding the physiological importance of "high affinity" ouabain binding sites in neuronal function.

Significance to Biomedical Research and the Program of the Institute: The psychotherapeutic agents described in these studies are among the most widely prescribed. Understanding the mechanisms by which these compounds exert their pharmacologic actions are of fundamental importance to a better understanding of epilepsy, anxiety-neuroses, sleep disorders, depression, and obesity. Furthermore, these studies can provide valuable information leading to the development of more efficacious therapeutic agents which lack major side effects.

Proposed Course of Project: The anxiogenic actions of certain benzodiazepine antagonists will be fully characterized in primates, with the ultimate goal of a standardized procedure for the chemical production of anxiety. Following establishment of anxiogenic regimens, the interaction of such compounds in other related behaviors (e.g. aggression) will be examined. The neurochemical mechanisms of strain differences in seizure susceptibility to certain β -carbolines will be examined. Benzodiazepine antagonists (e.g. RO 15-1788 and CGS-8216) will be tested for somnolytic actions (similar to 3-HMC), and any differences in action will be neurochemically studied. The use of both genetic mutants (such as the ob/ob mouse) and neuroanatomic lesions will be used in further characterizing the binding of [^3H] amphetamine to hypothalamic membranes. The role of imipramine binding sites in depressive illness will be further examined, including postmortem studies of suicide victims as well as unipolar and bipolar depressives. The recent development of a reliable radioreceptor assay for phencyclidine binding to brain tissue in vitro will lead to studies using chemically modified phencyclidine derivatives in an attempt to determine the physiologic (if any) role of these sites.

Miscellaneous: Co-chairperson, Pharmacology of Benzodiazepines, Symposium held at NIH, April 1982; editor, "Pharmacology of Benzodiazepines", MacMillan Press, Ltd., London, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00422-11 LCS |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Neuropharmacology of Circadian Rhythms | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: J.S. Takahashi, Research Associate LCS NIMH M. Zatz, Medical Officer (Research) LCS NIMH | | |
| Others: A. Eskin, Department of Biology, University of Houston | | |
| COOPERATING UNITS (if any) Department of Biology, University of Houston, Houston, Texas | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Pharmacology | | |
| INSTITUTE AND LOCATION NIMH ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.5 | PROFESSIONAL: 1.5 | OTHER: 0.0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> Circadian rhythms and environmental lighting regulate a number of endocrine and behavioral functions. The chick <u>pineal gland</u> and the <u>eye</u> of Aplysia remain rhythmic and responsive to light in vitro. Several lines of evidence in each of these systems indicate that <u>cyclic AMP</u> is involved in the generation and regulation of circadian rhythmicity. </p> | | |

Project Description:

Objectives: To elucidate the biochemical mechanisms and neuropharmacology of circadian rhythms.

Methods: Biochemical, pharmacologic, surgical, culture, radio-immunologic, and radioenzymatic techniques.

Major Findings: The avian pineal gland contains a circadian oscillator and a photoreceptor which regulate the synthesis and secretion of the hormone melatonin. Cultured glands express circadian rhythms in serotonin N-acetyltransferase activity and in cyclic AMP and cyclic GMP levels. Light exposure of the gland during the subjective night reduces the levels of both nucleotides and, subsequently, the activity of serotonin N-acetyltransferase. The effects of pharmacological agents which selectively affect cyclic AMP or cyclic GMP indicate that it is cyclic AMP levels which mediate the effects of light and regulate enzyme activity.

The eye of Aplysia expresses a robust circadian rhythm of neural output in vitro. Serotonin and forskolin both stimulate adenylate cyclase activity in homogenates. Their effects on the circadian pacemaker, as reflected in the phase response curves produced in intact eyes, are identical. Thus, serotonin acts on the circadian pacemaker via its activation of adenylate cyclase.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, temperature, and other physiologic functions. Elucidation of the mechanisms generating and regulating circadian rhythms are of broad clinical and biologic interest.

Proposed Course of Project: Mechanisms generating and regulating circadian rhythms and the effects of light will be explored further.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00425-06 LCS |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Peripheral and Central Catecholamines in Hypertension and Stress | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: J.M. Saavedra, Medical Officer (Psychiatry) LCS NIMH | | |
| Others: C. Chevillard, Guest Worker LCS NIMH J. Fernandez-Pardal, Guest Worker LCS NIMH N. Barden, Guest Worker LCS NIMH J. Furness, Guest Worker LCS NIMH C.M. Ferrario, Head, Hypertension Section K.B. Brosnihan, Research Assistant, Hypertension Section H. Holcomb, Clinical Associate BPB NIMH | | |
| COOPERATING UNITS (if any) Cardiovascular Research Unit, Cleveland, Ohio Biological Psychiatry Branch, NIMH | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Pharmacology | | |
| INSTITUTE AND LOCATION NIMH ADAMHA NIH, Bethesda, Maryland, 20205 | | |
| TOTAL MANYEARS: 6.0 | PROFESSIONAL: 6.0 | OTHER: 0.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> This project was initiated to study the role of <u>Catecholamines</u> in the <u>central</u> and <u>peripheral</u> system in <u>experimental</u> and <u>genetic</u> <u>hypertension</u> and <u>stress</u>, and has now been extended to study the participation of other <u>biogenic amines</u>, <u>estrogens</u>, and <u>prostaglandins</u> in the <u>central</u> regulation of <u>neurovegetative functions</u>. <u>Central catecholamines</u>, <u>histamine</u>, <u>serotonin</u> and several <u>neuropeptides</u> are involved in the regulation of <u>spontaneous</u> and <u>sodium-sensitive genetic hypertension</u>. There are specific and selective changes in <u>catecholamine metabolism</u> in <u>hypothalamic</u> and <u>brain stem nuclei</u> in <u>genetic</u>, <u>sodium dependent hypertension</u> in the <u>rat</u>, and in <u>sodium depleted dogs</u>. There are large changes in <u>catecholamine metabolism</u> in the <u>heart</u> and <u>adrenal medulla</u> of <u>genetic</u>, <u>sodium sensitive hypertensive rats</u>. Specific <u>binding</u> of <u>prostaglandin E₂</u> has been described in <u>rat brain membranes</u>. </p> <p> <u>Electrical stimulation</u> of selective <u>cerebellar nuclei</u> produces increases in <u>plasma catecholamines</u>. </p> | | |

Continuation

Names, Laboratory and Institute Affiliations, and Titles of Principal Investigators and All Other Professional Personnel Engaged on the Project

Others (continued): N. Contell, Research Assistant, BPB NIMH
 W. Pettinger, Professor, Department of Pharmacology,
 Southwestern University, Texas
 D. Reis, Professor, Department of Neurology, Cornell
 University, New York

Project Description:

Objectives: To study the role of central and peripheral catecholamines, other biogenic amines, prostaglandins and peptides in hypertension and stress.

Methods Employed: Anatomical: Microdissection of brain nuclei.
 Surgical: lesions of brain nuclei by specific neurotoxins and knife cuts.
 Surgical baroreceptor deafferentation. Biochemical: enzymatic-isotopic micromethods for the measurement of biogenic amines, their metabolites, and related enzymes.

Major Findings:

Brain catecholamines in sodium depletion - Specific alterations in noradrenaline metabolism occur in the area postrema of sodium depleted dogs.

Brain catecholamines in genetic hypertensive rats - Specific alterations in dopamine, noradrenaline and adrenaline occur in specific nuclei of the brain stem and hypothalamus of the sodium sensitive, genetic hypertensive rat.

Amines in Pituitary gland - Estrogen treatment profoundly effects the metabolism of catecholamines in the neurointermediate lobe of ovariectomized rats.

The turn over of histamine is very fast in the neural pituitary. Histamine levels are selectively changed in posterior pituitary gland of rats lacking vasopressin.

Prostaglandins in the rat brain and pituitary - There is a specific binding of prostaglandin E_2 to rat brain membranes, and especially the hypothalamus and amygdala as well as the posterior pituitary.

Prostaglandin D_2 (PGD₂) is quantitatively the most important prostaglandin to be formed by brain tissue after prolonged incubation. The amounts of PGD₂ are low, however, where brain tissues are immediately frozen after death.

Heart catecholamines - Profound changes in metabolism occur in the heart of genetic, sodium sensitive, hypertensive rats, indicating increased turnover of dopamine, adrenaline and noradrenaline in these rats.

Significance to Biomedical Research: The studies on biogenic amines and the central regulation of blood pressure and stress could help to explain some aspects of the role of specific brain structures in neuroendocrine functions, and also to further explain some basic aspects of the physiology of these brain transmitters.

Specific drugs which interfere with the neurotransmitter metabolism could be developed for application to the therapy of neuroendocrine disorders. As an example, inhibitors of adrenaline synthesis can lower blood pressure and are now being studied for their possible application to human pathological states.

Proposed Course of Project: Further studies will be conducted to determine the characteristics of the adrenaline-forming enzymes in brain, including specific lesions and studies with specific PNMT antibodies.

The role of catecholamines, serotonin, histamine, prostaglandins, estrogens and neuropeptides in genetic hypertension and stress will be studied with emphasis in the determination of amine metabolites, and will be focused in brain stem, hypothalamic and limbic system areas as well as in the pituitary gland.

Studies will continue to further characterize the changes in peripheral (heart, pineal, pituitary and kidney) and central catecholamines in spontaneous and sodium sensitive genetic hypertension.

The effects of estrogen on pituitary catecholamine metabolism will also be further analyzed.

Publications:

Brosnihan, K.B., Ferrario, C.M., Saavedra, J.M., and Smeby, R.R.: Central and peripheral relationships between renin activity and sympathetic nervous system during sodium depletion. J.P. Buckley and C.M. Ferrario (eds.): Central Nervous System Mechanisms in Hypertension. New York, Raven Press, 1981, pp. 385-395.

Brosnihan, K.B., Ferrario, C.M., Saavedra, J.M. and Speth, R.C.: Catecholamines and serotonin in the area postrema of normal and sodium-depleted dogs. Hypertension 3: 151-154, 1981.

Barden, N., Chevillard, C. and Saavedra, J.M.: Estradiol treatment decreases type A and increases type B monoamine oxidase in specific brain stem areas and cerebellum of ovariectomized rats. Brain Res 222: 177-181, 1981.

Barden, N., Chevillard, C. and Saavedra, J.M.: Twenty-four hour rhythm in monoamine oxidase activity in specific areas of the rat brain stem. Brain Res 223: 205-209, 1981.

Dray, F., Malet, C., Saavedra, J.M. and Scherrer, H.: Specific binding of [3 H] prostaglandin E_2 to rat brain membranes and synaptosomes. Brain Res 236: 227-233, 1982.²

Saavedra, J.M.: Brainstem epinephrine in genetic and experimental hypertension in the rat. H. Villarreal (ed.): Hypertension. New York, John Wiley & Sons, Inc. 1981, pp. 219-222.

Correa, F.M.A., Fernandez-Pardal, J. , Furness, J.B., Guicheney, P., McCarty, R., Rouot, B. and Saavedra, J.M.: Heart catecholamines in genetic hypertension. Delius, W., Gerlach, E., Grobecker, H. and Kubler, W. (eds.): Catecholamines and the Heart. Berlin, Springer-Verlag, 1981, pp.92-106.

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|---|--|---|-----|-------------------------------|----------|--|--|----------|--|------------------------|----------|--|--|--|---------|------------------------------|---------|--|----------------------------|------------|--|--|-----------|--|--|----------|--|--|-----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00427-05 LCS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Phospholipid Methylation and Signal Transduction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 60%;">F. Hirata, Visiting Scientist</td> <td style="width: 30%;">LCS NIMH</td> </tr> <tr> <td></td> <td>J. Axelrod, Chief, Section on Pharmacology</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>T. Notsu, Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Others:</td> <td>T. Hoffman, Senior Scientist</td> <td>LID NCI</td> </tr> <tr> <td></td> <td>Y. Ito, Visiting Scientist</td> <td>ORDA NIAID</td> </tr> <tr> <td></td> <td>M. Nirenberg, Chief, Lab. of Biomedical Genetics</td> <td>LBG NHLBI</td> </tr> <tr> <td></td> <td>R. Siraganian, Chief, Section on Clinical Immunology</td> <td>LMI NIDR</td> </tr> <tr> <td></td> <td>M. Vaughan, Chief, Laboratory of Cellular Metabolism</td> <td>LCM NHLBI</td> </tr> </table> | | | PI: | F. Hirata, Visiting Scientist | LCS NIMH | | J. Axelrod, Chief, Section on Pharmacology | LCS NIMH | | T. Notsu, Guest Worker | LCS NIMH | | | | Others: | T. Hoffman, Senior Scientist | LID NCI | | Y. Ito, Visiting Scientist | ORDA NIAID | | M. Nirenberg, Chief, Lab. of Biomedical Genetics | LBG NHLBI | | R. Siraganian, Chief, Section on Clinical Immunology | LMI NIDR | | M. Vaughan, Chief, Laboratory of Cellular Metabolism | LCM NHLBI |
| PI: | F. Hirata, Visiting Scientist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | J. Axelrod, Chief, Section on Pharmacology | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | T. Notsu, Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Others: | T. Hoffman, Senior Scientist | LID NCI | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Y. Ito, Visiting Scientist | ORDA NIAID | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | M. Nirenberg, Chief, Lab. of Biomedical Genetics | LBG NHLBI | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | R. Siraganian, Chief, Section on Clinical Immunology | LMI NIDR | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | M. Vaughan, Chief, Laboratory of Cellular Metabolism | LCM NHLBI | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) LID/NCI; LBG and LCM/NHLBI; LDBA and LMI/NIDR; ORDA/NIAID Division of Immunology, Johns Hopkins University, Baltimore, Maryland; Department of Biochemistry, New York State Univeristy, New York | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Pharmacology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH ADAMHA NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 5.0 | PROFESSIONAL: 5.0 | OTHER: 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Receptor-mediated cascade of <u>phospholipid metabolism</u> was further analyzed. This phospholipid cascade involves interaction of receptors with specific ligands, activation of phospholipid methylation, influx of Ca^{2+} , activation of kinases, phosphorylation of phospholipase inhibitory protein (lipomodulin), activation of phospholipases, release of arachidonate. Lipomodulin has been purified from conditioned media of neutrophils treated with glucocorticoids and a monoclonal antibody against this protein has been isolated. This protein can inhibit arachidonate release from various cells and anti-lipomodulin antibody can block the effects of lipomodulin. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Names, Laboratory and Institute Affiliations, and Titles of Principal Investigators and all Other Professional Personnel Engaged on the Project

Others (Continued):

K. Ishizaka, Professor of Immunology, Johns Hopkins University

Project Description:

Objectives: To study the role of phospholipid metabolism in the receptor-mediated signal transduction across biomembrane.

Methods Employed: Enzymatic, radiometric, immunological and pharmacological techniques.

Major Findings: We have previously shown that many receptors, if not all, interact with methyltransferase of phospholipids and that stimulation of receptors result in increase of phospholipid methylation. To establish such interaction, β -adrenergic receptors were partially purified and transplanted into human red blood cell ghosts which have the methyltransferases, but not β -adrenergic receptors. The ghost membranes responded to β -agonists as measured by stimulation of phospholipid methylation. The idea that stimulation of phospholipid methylation by ligands requires receptors has been further established by the findings that anti-Ig E receptor antibody can increase phospholipid methylation in plasma membrane fraction but not in mitochondrial fraction from rat mast cells, although both fractions have the methyltransferase activities. Ig E receptors are primarily located in the plasma membranes.

Phospholipase inhibitory protein has been isolated from the conditioned media of rabbit neutrophils incubated with glucocorticoids. Purified lipomodulin can inhibit arachidonate release from fibroblast stimulated by bradykinin (hormone), from lymphocytes stimulated by Con A (mitogen) and from neutrophils stimulated by fMet-Leu-Phe (chemoattractant).

The inhibition of arachidonate release from these cells can be observed by the treatment with glucocorticoids such as dexamethasone. The monoclonal antibody against lipomodulin reversed the effects of both lipomodulin and glucocorticoids. These results suggest that lipomodulin mimic the action of glucocorticoid on the suppression of arachidonate release from various cells.

Significance to Biomedical Research: The cascade of phospholipid metabolism appears to be a fundamental mechanism; lipomodulin especially seems to play an important role in the signal transduction. Glucocorticoids are well known to regulate various receptor functions in the presence of the second hormone and neurotransmitter. Lipomodulin can mimic the glucocorticoids, suggesting its application for allergy, rheumatic disease, differentiation of cancer cells and some neurological diseases.

Proposed Course of Project: Lipomodulin will be purified in a large quantity and will be applied for in vivo and in vitro tests to examine its

steroid-like action. Radioimmunoassay will be established to measure lipomodulin level in which glucocorticoids play an important role.

Publications:

Axelrod, J., and Hirata, F.: Phospholipid methylation and membrane function. Ann. N.Y. Sci. 373: 51-53, 1981.

Axelrod, J., and Hirata, F.: Phospholipid methylation and receptor mediated transmission of biological signals through membranes. In Birdsall, N.J.M. (Ed.): Drug Action: Drug Receptors and their Effectors. London, McMillan Publisher Ltd., 1981, pp. 51-58.

Axelrod, J., and Hirata, F.: Phospholipid methylation and the receptor induced release of histamine from cells. Trends in Pharmacol. Sci., 1982, in press.

Axelrod, J., Hirata, F., Crews, F.T., Ishizaka, T., Ishizaka, K., McGivney, A., and Siraganian, R.P.: Lipids and the receptor-mediated release of histamine from cells. Stjarne, L., Lagercrantz, H., Hedqvist, P., and Wennmalm, A. (Eds.): Chemical Neurotransmission 75 years. London, Academic Press Inc., 1981, pp.319-328.

Axelrod, J., Hirata, F., Crews, F.T., Ishizaka, T., Ishizaka, K., McGivney, A., and Siraganian, R.P.: Lipids and the receptor mediated release of histamine. In Yoshida, H., Hagihara, Y. and Ebashi, S. (Eds.): Advances in Pharmacology and Therapeutics II, Vol. 2, Neurotransmitters Receptors, New York, Pergamon Press, 1982, pp57-67.

Bareis, D.L., Hirata, F., Axelrod, J. and Schiffmann, E.: Phospholipid metabolism, calcium flux and the receptor mediated induction of chemotaxis in rabbit neutrophils. J. Cell Biol., 1982, in press.

Bougnoux, P., Hirata, F., Timonen, T., and Hoffman, T.: Effects of interferon on phospholipid metabolism in human peripheral blood cells. In Resh, K., and Hirschner (Eds.): Mechanism of Lymphocyte Activation, Amsterdam, Elsevier/North Holland, 1981, pp. 577-580.

Crews, F.T., Morita, Y., McGivney, A., Hirata, F., Siraganian, R.P., and Axelrod, J.: IgE mediated histamine release in rat basophilic leukemia cells: receptor activated phospholipid methylation, Ca^{2+} flux, and release of arachidonic acid. Arch. Biophys. Biochem. 212: 561-571, 1981.

Gagnon, C., Kelley, S., Manganiello, V., Vaughan, M., Strittmatter, W.J., Hoffman, A., and Hirata, F.: Modification of calmodulin function by enzymatic carboxyl methylation. Nature 291: 515-516, 1981.

Crews, F.T., Camacho, A., Phillips, I., Calderini, G., Hirata, F., Axelrod, J., McGivney, A., and Siraganian, R.: Effects of membrane fluidity on mast cell and nerve cell function. Proc. of the VII International Congress of Neurochemistry, Birmingham, England, 1982, in press.

Daeron, M., Sterk, A.R., Hirata, F., and Ishizaka, T.: Biochemical analysis of glucocorticoids-induced inhibition of IgE-mediated histamine release from mouse mast cells. J. Immunol., in press.

Hirata, F.: Overviews on phospholipid methylation. In Usdin, E., Borchart, R., Creveling, C. (Eds.): Transmethylation, New York, MacMillan Press, 1982, in press.

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Hirata, F., Cr ews, F.T., Axelrod, J., McGivney, A., Siraganian, R.P., Ishizaka, T., and Ishizaka, K.: Biochemical mechanism of histamine release from rat peritoneal mast cells and rat basophilic leukemia cells. Proceedings of the Satellite Symposium on Metabolism and Biology of Histamine, Okayama, Japan, 1981, in press.

Siraganian, R.P., McGivney, A., Barsumian, E.L., Crews, F.T., Hirata, F., and Axelrod, J.: Use of variants of the rat basophilic leukemia cell line for study of histamine release. Fed. Proc., 1982, in press.

Toyoshima, S., Hirata, F., Axelrod, J., Beppu, M., Osawa, T., and Waxdal, M.J.: Relationship between phospholipid methylation and calcium influx in murine lymphocytes stimulated with native and modified concanavalin A. Mol. Immunol., 1982, in press.

Toyoshima, S., Iwata, M., Hirata, F., Axelrod, J., Osawa, T., and Waxdal, M.J.: Phospholipid methylation: a possible role in lymphocyte mitogenesis. Mol. Immunol., 1982, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00428-03 LCS | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Protein Carboxyl Methylation: A Post Translational Modifier of Protein Function | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 60%;">Y. Kloog, Fogarty International Fellow</td> <td style="width: 25%;">LCS NIMH</td> </tr> <tr> <td></td> <td>J.M. Saaavedra, Medical Officer (Psychiatry)</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>J. Axelrod, Chief, Section on Pharmacology</td> <td>LCS NIMH</td> </tr> <tr> <td>Others:</td> <td>D. Flynn, Depart. of Pharmacology, University of Miami</td> <td></td> </tr> <tr> <td></td> <td>I. Spector, Guest Worker</td> <td>LBG NHLBI</td> </tr> </table> | | | PI: | Y. Kloog, Fogarty International Fellow | LCS NIMH | | J.M. Saaavedra, Medical Officer (Psychiatry) | LCS NIMH | | J. Axelrod, Chief, Section on Pharmacology | LCS NIMH | Others: | D. Flynn, Depart. of Pharmacology, University of Miami | | | I. Spector, Guest Worker | LBG NHLBI |
| PI: | Y. Kloog, Fogarty International Fellow | LCS NIMH | | | | | | | | | | | | | | | |
| | J.M. Saaavedra, Medical Officer (Psychiatry) | LCS NIMH | | | | | | | | | | | | | | | |
| | J. Axelrod, Chief, Section on Pharmacology | LCS NIMH | | | | | | | | | | | | | | | |
| Others: | D. Flynn, Depart. of Pharmacology, University of Miami | | | | | | | | | | | | | | | | |
| | I. Spector, Guest Worker | LBG NHLBI | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Department of Pharmacology, School of Medicine, University of Miami, Miami, Florida | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | |
| SECTION Section on Pharmacology | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH ADAMHA NIH Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2.0 | PROFESSIONAL: 2.0 | OTHER: 0.0 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | |
| <p>Enzymatic <u>carboxyl methylation</u> of proteins is a post translational modification. By <u>changing</u> the structure and properties of membrane proteins, carboxyl methylation provides an important regulatory mechanism. Receptors and ion channel proteins being molecules that transmit information across the cell membrane are the obvious targets for regulating modification.</p> <p>The possible function of carboxyl methylation of membrane proteins was studied in neuroblastoma cell line NIE-115. These cells were found to be enriched in the enzyme protein carboxyl methylase (PCM). The enzyme is highly localized in the cytosol while its substrates, the methyl acceptor proteins are present mainly in the particulate fraction. These data suggest that PCM does function as a regulator of membrane bound proteins. Indeed, in the NIE-115 cells we found an increase in enzyme activity (two-fold) and a dramatic increase in methyl acceptor proteins (five-fold) as the cells</p> | | | | | | | | | | | | | | | | | |

undergo morphological and functional differentiation. The time course of this increase in carboxyl methylation paralleled the development of electrophysiological response of these cells. These data show a close relationship between the development of carboxyl methylation and membrane ion translocation, similar to our observation on methylation of the membrane bound acetylcholine receptor from Torpedo electric organ.

Protein carboxyl methylation has been associated also with exocytotic release. The latter being dependent on depolarization of the cells' membrane and Ca^{++} ions movements. In the posterior pituitary lobe, exocytotic release of vasopressin, oxytocin and their corresponded neurophysins occur upon depolarization. We have studied carboxyl methylation of proteins in posterior pituitary lobes of Brattleboro rats with diabetes insipidus which lack vasopressin and vasopressin-neurophysin. In these rats methyl acceptor protein capacity was found to be 80% lower than in control rats while PCM activity is about 40% higher. The low methyl acceptor protein capacity is due to the low methylation of 11K daltons protein reflecting the absence of vasopressin-neurophysin which is a major substrate in the posterior pituitary. The increased enzyme activity can be attributed to the hyperactivity of the posterior pituitary of the Brattleboro rat. The data show the close relationship between the activity of posterior lobe and carboxyl methylation.

Project Description:

Objectives: To find a relationship between enzymatic carboxymethylation and the function of the acetylcholine receptor and voltage sensitive ion channels and exocytotic release.

Methods Employed: Enzymatic, pharmacologic

Major Findings: Methyl acceptor membrane bound proteins in neuroblastoma cells increase as these cells undergo morphological and functional differentiation. The time course of the increase in carboxyl methylation parallels the development of electrophysiological response of the cells.

In the neurosecretory posterior pituitary several peptides are methylated among them the neurophysins. A decrease in carboxyl methylation in posterior lobes of Brattleboro rats was found and is attributed to the lack of vasopressin-neurophysin in these rats.

Significance to Biomedical Research: Carboxyl methylation may prove to be a key regulatory step in the activity of a number of enzymes and receptors by reducing negative charges on membrane proteins.

Proposed Course of Project: The identification of methyl acceptor proteins will be delineated and the functional consequences of methylation will be investigated.

Publications:

Flynn, D., Kloog, Y., Potter, L.T., and Axelrod, J.: Enzymatic methylation of the membrane bound nicotinic acetylcholine receptor. J. Biol. Chem. 1982, in press.

Kloog, Y., and Axelrod, J.: Protein carboxyl methylation in Eukaryotes. Acta Physiol. Scand. [Suppl.], 1982, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00429-03 LCS |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biosynthesis of Nonpolar Methylated Lipids | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: M. Zatz, Medical Officer (Research) S. Markey, Pharmacologist | | LCS NIMH LCS NIMH |
| Other: S. Engelsen, Guest Worker | | LCS NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Pharmacology | | |
| INSTITUTE AND LOCATION NIMH ADAMHA NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.3 | PROFESSIONAL: 1.0 | OTHER: 0.3 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> The newly discovered <u>methylation</u> of free fatty acids by <u>S-adenosyl-methionine</u> was characterized in human <u>red blood cells</u>. The reaction occurs in whole cells on the cytoplasmic side of the <u>plasma membrane</u>. Biosynthesis of fatty acid methyl esters is one of the consequences of <u>phospholipase A₂</u> activation in plasma membranes. </p> <p> The biosynthesis of another nonpolar methylated product was discovered upon incubation of lung membranes with <u>oleoylcoenzyme A</u> and <u>S-adenosyl-methionine</u>. This product was identified as <u>S-methyl-oleoylcysteamide</u> and appears to be formed by cell membranes, including plasma membranes, after amidase cleavage of <u>oleoylcoenzyme A</u>. </p> | | |

Project Description:

Objectives: To identify the nonpolar lipids formed from S-adenosylmethionine and to investigate their biochemistry and physiology.

Methods Employed: Biochemical, chromatographic, chemical, pharmacologic, and radioactive trace techniques.

Major Findings: Nonpolar methylated products comprise about 50% of the radioactive material extractable into chloroform/methanol after incubation of human red cell membranes with S-[methyl-³H]adenosylmethionine. One of these nonpolar products is fatty acid methyl ester. The enzyme which synthesizes fatty acid methyl ester had an apparent K_m for S-adenosylmethionine of about 0.6 μ M and a V_{max} of about 0.6 pmol/mg protein per 30 min. Half-maximal activity was achieved upon addition of about 20 μ M sodium oleate. Of the fatty acids tested, sodium oleate most effectively increased activity (6-fold). Fatty acid methylation takes place in intact cells incubated with [methyl-³H]methionine on the cytoplasmic side of the plasma membrane. Incubation of cells with melittin, a potent membrane phospholipase A₂ activator from bee venom, increased fatty acid methylation several-fold. Fatty acid methylation appears to be one of the consequences of phospholipase action in plasma membranes.

Incubation of membranes from lung, kidney, liver, spleen, or human red cells with radioactive S-adenosylmethionine and oleoylcoenzyme A results in the formation of a previously unidentified lipid. This product was identified as S-methyl-oleoylcysteamide by mass spectrometry after isolation by extraction, TLC, HPLC, and GC. Biosynthesis of this product occurs via an enzymatic process. The reaction occurs in microsomal membranes and plasma membranes. The product may be formed from oleoylcoenzyme A after cleavage by an amidase, by rearrangement and S-methylation.

Significance to Biomedical Research: The role of membrane lipids in cellular function, particularly in signal transduction, is currently being recognized and elucidated. Methylation of fatty acids and thiols by plasma membranes may play a dynamic role in cellular function.

Proposed Course of Project: The biochemistry and physiology of nonpolar lipid methylation will be investigated.

Publications:

Zatz, M., Dudley, P.A., Kloog, Y.: Nonpolar lipid methylation: Biosynthesis of fatty acid methyl esters by rat lung membranes using S-adenosylmethionine. J. Biol. Chem. 256: 10028-10032, 1981.

Kloog, Y., Zatz, M., Rivnay, B., Dudley, P.A. and Markey, S.P.: Nonpolar lipid methylation: Identification of nonpolar methylated products synthesized by rat basophilic leukemia cells, retina, and parotid. Biochem. Pharm. 31: 753-760, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00433-02 LCS |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Role of Neuropeptides in Neuroendocrine Regulation | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J.M. Saavedra, Medical Officer (Psychiatry) LCS NIMH Others: C. Chevillard, Guest Worker LCS NIMH J. Fernandez-Pardal, Guest Worker LCS NIMH Y. Kloog, PHS International Fellow LCS NIMH F. Dray, Head, Unit on RIA, Institute Pasteur, France C. Rougeot, Research Assistant, Unit on RIA, Institute Pasteur, France K. Gerozitssis, Established Investigator, Unit on RIA, Institute Pasteur, France A. Negro-Vilar, Assoc. Prof., Dept. Physiol., Southwestern Univer., Texas | | |
| COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, NIMH Biological Psychiatry Branch, NIMH Unit on RIA, Institute Pasteur, France | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Pharmacology | | |
| INSTITUTE AND LOCATION NIMH ADAMHA NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 5.0 | PROFESSIONAL: 5.0 | OTHER: 0.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project was initiated to study the role of <u>brain neuropeptides</u> in <u>genetic hypertension</u> and <u>stress</u> and has now been extended to study the <u>interrelationship</u> between different <u>neuropeptides</u> and their role in <u>neuroendocrine regulation</u> . <u>Angiotensin-converting enzyme (ACE)</u> activity is heterogeneously distributed in the rat <u>brain</u> , with highest activity in the <u>subfornical organ</u> . <u>Spontaneously hypertensive rats</u> show specific changes in ACE in <u>intermediate</u> and <u>anterior pituitary</u> lobes, and in specific nuclei of the <u>brain stem</u> . Changes in <u>somatostatin</u> occur in the <u>pituitary</u> and selective <u>hypothalamic nuclei</u> of rats lacking vasopressin. | | |

Names, Laboratory and Institute Affiliations, and Titles of Principal Investigators and All Other Professional Personnel Engaged on the Project

Others (Continued): P. Gross, Visiting Scientist, Laboratory of Cerebral Metabolism, NIMH
M. Kutina, Visiting Scientist, Laboratory of Cerebral Metabolism, NIMH
H. Holcomb, Clinical Associate, Biological Psychiatry Branch, NIMH

Cooperating Units (Continued):

Department of Physiology, Southwestern University, Texas

There are alterations in protein carboxylmethylation in rats lacking vasopressin.

Project Description:

Objectives: To study the functions of central neuropeptides and their role in neuroendocrine regulation.

Methods Employed: Anatomical: microdissection of brain nuclei and specific lesions by knife cuts; immunohistofluorescent techniques: Biochemical: radioimmunoassays of neuropeptides; high pressure liquid chromatography.

Major Findings:

Central angiotensin system. Specific circumventricular nuclei, such as the subfornical organ, contain very high ACE activity. Alterations in ACE activity occur in specific brain stem nuclei of rats lacking vasopressin.

Neuropeptides in hypertension. Spontaneously hypertensive rats present high ACE activity in the intermediate pituitary lobe, and low ACE in the anterior lobe, plasma, and specific brain stem nuclei.

Control of vasopressin secretion. In vitro experiments with posterior pituitary homogenates and with whole posterior pituitaries demonstrate the presence of several endogenous proteins which are substrates of protein carboxylmethylase. One of these proteins is absent in rats lacking vasopressin and neuophysin-vasopressin.

Significance to Biomedical Research: The study of the interactions between different neuropeptides and between neuropeptides and catecholamines will clarify part of the role of these compounds in the regulation of blood pressure and other related neuroendocrine functions. The use of drugs which interfere with the metabolism and function of neuropeptides could result in future therapeutic advantages in the treatment of neuroendocrine disorders.

Proposed Course of Project: We plan to study further the physiological and pathophysiological interactions between the vasopressin and angiotensin

systems in brain and pituitary gland, and the interrelations between vasopressin, somatostatin, opioid peptides and biogenic amines in the posterior and intermediate lobes of the pituitary gland.

The role of neuropeptides in the functions of the posterior and intermediate lobes of the pituitary gland will be studied by a combination of lesions, including pituitary stalk sections, the use of specific antibodies, drug treatments, and physiological manipulations.

We also plan to study further the mechanism for release of vasopressin from the posterior pituitary, and specifically the role of protein carboxymethylation in neurophysin-vasopressin metabolism, storage and release.

Publications:

Barden, N., Chevillard, C., and Saavedra, J.M.: Diurnal variations in rat posterior pituitary catecholamine levels. Neuroendocrinology 34: 148-150, 1982.

Chevillard, C., and Saavedra, J.M.: High antihypertensive enzyme activity in the neurohypophysis of Brattleboro rats. Science 216: 646-647, 1982.

Chevillard, C., and Saavedra, J.M.: Distribution of antihypertensive enzyme activity in specific areas of the rat brain stem. J. Neurochem. 38: 281-284, 1982.

Saavedra, J.M.: Central biogenic amines and neuropeptides in genetic hypertension. In Buckley, P. and Ferrario, C.M. (Eds.): Central Nervous System Mechanisms in Hypertension. New York, Raven Press, 1981, pp. 129-139.

Saavedra, J.M.: Spontaneously (genetic) hypertensive rats: Naloxone-reversible and propranolol-reversible decrease in pain sensitivity. Experientia 37: 1002-1003, 1981.

Saavedra, J.M.: Biogenic amines and neuropeptides play a role in the central regulation of genetic hypertension. In Worcel, M., Bonvalet, J.P., Langer, S.J., Menard, J., and Sassard, J. (Eds.): New Trends in Arterial Hypertension, INSERM Symposium 17, Amsterdam, Elsevier/North-Holland Biomedical Press, 1981, pp 11-23.

Saavedra, J.M., and Chevillard, C.: Angiotensin-converting enzyme is present in the subfornical organ and other circumventricular organs of the rat. Neurosci. Lett. 29: 123-127, 1982.

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|--|--|---|-----|--|----------|--|--------------------------------|----------|--|-----------------------------|----------|--|--------------------------|----------|--|--|----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00434-01 LCS | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Cellular and Molecular Mechanisms of ACTH Secretion from Mouse Pituitary Tumor Cells | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 60%;">J. Axelrod, Chief, Section on Pharmacology</td> <td style="width: 30%;">LCS NIMH</td> </tr> <tr> <td></td> <td>S. Heisler, Visiting Scientist</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>V. Hook, Research Associate</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>T. Reisine, Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Y. Kloog, Fogarty International Fellow</td> <td>LCS NIMH</td> </tr> </table> | | | PI: | J. Axelrod, Chief, Section on Pharmacology | LCS NIMH | | S. Heisler, Visiting Scientist | LCS NIMH | | V. Hook, Research Associate | LCS NIMH | | T. Reisine, Guest Worker | LCS NIMH | | Y. Kloog, Fogarty International Fellow | LCS NIMH |
| PI: | J. Axelrod, Chief, Section on Pharmacology | LCS NIMH | | | | | | | | | | | | | | | |
| | S. Heisler, Visiting Scientist | LCS NIMH | | | | | | | | | | | | | | | |
| | V. Hook, Research Associate | LCS NIMH | | | | | | | | | | | | | | | |
| | T. Reisine, Guest Worker | LCS NIMH | | | | | | | | | | | | | | | |
| | Y. Kloog, Fogarty International Fellow | LCS NIMH | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Unit of Cellular and Molecular Bioregulation, Laval University, Quebec, Canada | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | |
| SECTION Section on Pharmacology | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH ADAMHA NIH Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 3.5 | PROFESSIONAL: 3 | OTHER: 0 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The mouse <u>pituitary tumor cell line AtT-20/D16-16</u> is an excellent model to study the physiological regulation of <u>synthesis, storage and secretion</u> of <u>ACTH</u> and <u>beta-endorphin</u>. Initial studies focussed on the characterization of the secretory response to stimulants such as the recently available synthetic 41-residue <u>corticotropin-releasing factor (CRF)</u> and to antagonists such as <u>glucocorticoids</u>. The CRF secretory effect is <u>calcium-dependent</u> and rapid <u>cyclic AMP</u> synthesis is associated with hormonal excitation. </p> <p> CRF was found to stimulate <u>phospholipid methylation</u> in AtT-20 cells, a reaction which appears in other cell systems as an important transducing mechanism following hormone-receptor interaction. </p> <p> Post-translational modification of protein by <u>carboxylmethylation</u> has been implicated in exocytotic secretion in both endocrine and exocrine glands and similar operative mechanisms stimulated by CRF are found in the AtT-20 cell. </p> | | | | | | | | | | | | | | | | | |

Secretion of ACTH and beta-endorphin from AtT-20 cells appears to be under multifactorial regulation by both peptides and neurotransmitters. We have identified beta-adrenergic receptors in the AtT-20 cells by both binding and ACTH secretory studies and have also found that some CNS peptides such as VIP and somatostatin influence the secretory response to ACTH.

These observations of hormonal interaction will enable us to further dissect both cellular and molecular mechanisms involved in the transduction and effectuation of the physiological response in the AtT-20 cells and will eventually provide insight and understanding of the importance of the anterior pituitary in the pathogenesis of stress.

Projection Description:

Objectives: To investigate at the cellular and molecular level various aspects of the ACTH secretory pathway in the mouse clonal pituitary tumor cell line, AtT-20/D16-16.

Methods Employed:

Cell culture. Biochemical: radioimmunoassay of ACTH, and cyclic nucleotides; gel electrophoresis, enzyme assays, cellular subfractionation and characterization. Pharmacological: radioreceptor assays.

Major Findings:

Responsivity of AtT-20 to physiological regulators: Ovine corticotropin releasing factor (CRF) stimulates ACTH and beta-endorphin secretion. CRF analogues stimulate peptide release with the same order of potency in the tumor cells as in primary culture of anterior pituitary cells. Dexamethasone markedly inhibits CRF-stimulated and basal ACTH and beta-endorphin release. The response to CRF is preceded by rapid cycle AMP synthesis, and requires the presence of extracellular calcium.

Phospholipid methylation in AtT-20 cells: Phospholipid methylation in AtT-20 cells parallels the ACTH secretory phenomenon following CRF stimulation. The responses are dose and time dependent and inhibited by drugs which block either methylation of phospholipids or ACTH secretion.

Protein carboxylmethylation in AtT-20 cells: Protein carboxyl-methylation activity and ACTH secretion increase or decrease in parallel following pharmacological manipulation of the AtT-20 cells suggesting that the enzyme reaction is involved in exocytotic secretion of ACTH. Following CRF stimulation the AtT-20 cells synthesize a 13-15K substrate (identity unknown) which can be carboxylmethylated

Beta-Adrenergic receptors in AtT-20 cells: Beta-Adrenergic receptors in AtT-20 cells were characterized by their ability to stimulate the secretion of ACTH and to increase intracellular synthesis of cyclic AMP. These effects are stereospecific. Binding of the radioactive beta-adrenergic antagonist dihydroalprenolol was characterized in crude membrane preparations.

Other peptide receptors in AtT-20 cells: VIP stimulates ACTH secretion and its effects are additive with CRF; somatostatin is an antagonist of the CRF secretory response.

Significance to Biomedical Research: The study of the cellular and molecular interactions of different peptides and catecholamines on the AtT-20 cell will clarify the importance of these substances in the pathogenesis of stress-induced ACTH release.

Proposed Course of Project: We plan to determine whether CRF, beta-adrenergic agonists, VIP and somatostatin interact at the level of phospholipid methylation. Using this information we will investigate the enzymatic cascade which may be associated with stimulation of phospholipid methylation (phospholipase A₂ activation, Ca-ATPase). We will attempt to identify the protein substrate which is carboxylmethylated following CRF stimulation. We will study the influence of glucocorticoid treatment on the beta-adrenergic and CRF receptor responsivity in the AtT-20 cells.

Publications:

Hook, V.Y.H., Heisler, S., Sabol, S.L. and Axelrod, J.:
Corticotropin releasing factor stimulates adrenocorticotropin and beta-endorphin release from AtT-20 mouse pituitary tumor cells.
Biochem. Biophys. Res. Commun., 1982, in press.

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|---|--|---|-----------------|---------------------|---|---------|---------------------|--|--|----------------|-----------------------|--|--------------------|-----------------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00382-08 LCS | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Localization and Characterization of Brain Neuropeptides</p> | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">David M. Jacobowitz</td> <td style="width: 33%;">Chief, Histopharmacology Section LC³ NIMH</td> </tr> <tr> <td>OTHERS:</td> <td>Thomas L. O'Donohue</td> <td>Guest Worker (NIGMS Fellow) LCS NIMH NIGMS</td> </tr> <tr> <td></td> <td>John Olschowka</td> <td>Staff Fellow LCS NIMH</td> </tr> <tr> <td></td> <td>Clivel G. Charlton</td> <td>Guest Worker LCS NIMH</td> </tr> </table> | | | PI: | David M. Jacobowitz | Chief, Histopharmacology Section LC ³ NIMH | OTHERS: | Thomas L. O'Donohue | Guest Worker (NIGMS Fellow) LCS NIMH NIGMS | | John Olschowka | Staff Fellow LCS NIMH | | Clivel G. Charlton | Guest Worker LCS NIMH |
| PI: | David M. Jacobowitz | Chief, Histopharmacology Section LC ³ NIMH | | | | | | | | | | | | |
| OTHERS: | Thomas L. O'Donohue | Guest Worker (NIGMS Fellow) LCS NIMH NIGMS | | | | | | | | | | | | |
| | John Olschowka | Staff Fellow LCS NIMH | | | | | | | | | | | | |
| | Clivel G. Charlton | Guest Worker LCS NIMH | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Laboratory of Clinical Science</p> | | | | | | | | | | | | | | |
| SECTION <p style="text-align: center;">Histopharmacology</p> | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">3.3</td> <td style="text-align: center;">1.7</td> <td style="text-align: center;">1.6</td> </tr> </table> | | | TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | 3.3 | 1.7 | 1.6 | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | |
| 3.3 | 1.7 | 1.6 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Highly specific and sensitive radio-immunoassays coupled to HPLC and gel filtration techniques were utilized to identify <u>secretin</u> , <u>motilin</u> and <u>pancreatic polypeptide</u> immunoreactivity in the brain of the rat. <u>Immunocytochemical</u> studies revealed a wide distribution of pancreatic polypeptide (PP) immunoreactive cell bodies and nerve fibers in the rat central and peripheral nervous systems. A number of PP-immunoreactive cells were demonstrated to coexist with <u>catecholamine neurons</u> in the pons-medulla and sympathetic ganglia. Motilin-like immunoreactive nerve fibers and cell bodies were observed in the hypothalamus, preoptic areas and Purkinje cells of the cerebellum. Radioimmunological studies revealed a wide distribution pattern of secretin in the brain of the rat. A brain-pituitary pathway for secretin has also been identified. Intraventricular injection of secretin reduced open field activity and the number of novel-object approaches in rats. Secretin also decreased respiration rate in anesthetized rats and increased defecation in awake rats. Intravenous injection of secretin in anesthetized hydrated rats caused a dose-related antidiuretic effect. | | | | | | | | | | | | | | |

Project Description:

Objectives: 1) Identification of pancreatic polypeptide-like immunoreactive cell bodies and nerve fibers in the central and peripheral nervous systems; demonstration of PP release from nerve terminals after electrical stimulation; and demonstration of PP coexistence in catecholamine neurons in the central and peripheral nervous systems. 2) Identification of secretin-like pathways in the central nervous system of the rat and determination of the role of secretin on, and its binding characteristics in, central and peripheral target cells.

Methods Employed: 1) Secretin radioimmunoassay; 2) High pressure liquid chromatography (HPLC); 3) Microdissection of rat brain; 4) Immunocytochemistry of secretin and PP; 5) Electrical stimulation of peripheral nerves of the rat; 6) Monitor urine output, defecation, respiration and motor activity following intraventricular as well as intravenous injection of secretin.

Major Findings:

(A) Secretin. 1) Identification and characterization of secretin-like immunoreactivity (SLI) in the brain of rat. 2) Demonstrate that the SLI in the pituitary is localized predominantly in the neurointermediate lobe. In pituitary stalk-transected rats the SLI of the neurointermediate lobe was significantly decreased, while the SLI of the anterior lobe remained unchanged. These data suggest that a brain-pituitary pathway for secretin may exist. 3) Intracerebroventricular injection of secretin reduced open field activity and decreased the number of novel-object approaches in rat. Secretin also decreased respiration rate in anesthetized rats and increased defecation in awake rats. 4) The intravenous injection of secretin in anesthetized hydrated rats caused significant dose-related antidiuretic effects.

(B) Pancreatic Polypeptide. 1) Distribution of PP immunoreactive neurons and nerve fibers in the central nervous system. In the forebrain, PP immunoreactive cell bodies were observed in the cerebral cortex, olfactory tubercle, preoptic area and hypothalamus. The arcuate nucleus contained the largest numbers of PP neurons. Lesions of the arcuate nucleus (electrolytic, mono-sodium glutamate or gold thioglucose) resulted in a marked decrease in PP fibers in the preoptic and hypothalamic areas. Caudally, PP cells were found in the regions which contain catecholamine cell groups -- the locus coeruleus (A6), the nucleus tractus solitarius (A2) and in the region of the nucleus reticularis (A1). PP nerve fibers were widely distributed, but were especially dense in the following nuclei: accumbens, interstitialis stria terminalis, preoptic medialis, supra-chiasmaticus, periventricular thalamic and hypothalamic, paraventricular, dorso-medialis, tractus solitarius, parabrachialis dorsalis and the substantia gelatinosa trigemini. 2) Distribution of PP neurons and nerve fibers in the peripheral nervous system (PNS). PP immunoreactive cell bodies were observed in the sympathetic ganglia (superior cervical, middle cervical, stellate), pancreatic islets, secretory epithelium of the gut, submucosal and myenteric plexi, and the adrenal medulla. PP nerve fibers were distributed in the PNS in a pattern similar to that of the catecholamines. The vas deferens had a dense innervation; the sino-atrial node and musculature of the heart and gut wall had a moderate innervation; the vascular walls of most organs had a moderate to low innervation. 3) Coexistence of PP and catecholamine in neurons of the CNS and

PNS. The presence of PP cells in regions of the pons-medulla containing catecholamine neurons suggested the possibility of coexistence of those two transmitters. The immunocytochemical staining of adjacent 6 μ m thick sections for PP and dopamine-beta-hydroxylase demonstrated the presence of both in a proportion of the A6, A2 and A1 catecholamine cells. Lesions of the catecholamine axon terminals (knife cut of the ventral noradrenergic bundle, intraventricular 6-hydroxydopamine (6-OHDA)) failed to demonstrate a noticeable decrease in hypothalamic PP nerve terminals. Therefore, the hypothalamic projection of PP-containing catecholamine cells appears minor compared to the arcuate nucleus PP projections. In the PNS, PP appeared to be restricted solely to catecholamine neurons and nerve fibers. 6-OHDA lesions of the peripheral catecholamine axons resulted in a complete disappearance of PP-containing nerve fibers. The significance of PP in catecholamine neurons of both the CNS and PNS is unknown.

4) Release of PP after electrical stimulation of superior cervical ganglia (SCG) neurons. Prior studies have shown that norepinephrine (NE) is released from varicose terminals during high nerve impulse activity. To determine if PP is co-released with NE during stimulation, the right cervical sympathetic trunk was supramaximally stimulated for 20 or 40 min. The left trunk served as control. Immediately after stimulation, the animal was perfused and processed for immunocytochemistry. A few animals were pretreated with reserpine 5 hr prior to stimulation. On the stimulated side, the PP fibers of the eye and salivary gland were virtually depleted of their PP content as compared to control. These results suggest that PP coexists with a proportion of SCG catecholamine cells and that it is coreleased with NE during stimulation.

Significance to Biomedical Research and the Program of the Institute: The basic neuroanatomical, immunochemical, behavioral and physiological studies reported here lay the groundwork for a rational approach to studying the role of secretin and pancreatic polypeptide in central and peripheral nervous system physiology.

Proposed Course of the Project:

1) Further studies are in progress to determine the biochemical levels of PP within various regions of the brain. Biochemical levels will be determined for diurnal rhythm studies, regeneration studies, drug treatment studies and stress studies.

2) Modulatory effects of PP co-release with norepinephrine will be studied both biochemically and physiologically.

3) Study further the brain-pituitary secretin pathway in the rat with emphasis on: a) the specific source and origin of the secretin immunoreactivity, b) the physiology of pituitary secretin in terms of release mechanism, circadian rhythm and functions, c) the relationship of the brain-pituitary secretin system with the vasopressin-oxytocin system.

4) Immunocytochemical localization and microdistribution of SLI in rat brain.

Publications:

Olschowka, J.A., O'Donohue, T.L. and Jacobowitz, D.M.: The distribution of bovine pancreatic polypeptide-like immunoreactive neurons in rat brain.

Peptides 2: 309-331, 1981.

Jacobowitz, D.M. and Olschowka, J.A.: Bovine pancreatic polypeptide-like immunoreactivity in brain and peripheral nervous system: Coexistence with catecholaminergic nerves. Peptides, in press, 1982.

Jacobowitz, D.M. and Olschowka, J.A.: Coexistence of bovine pancreatic polypeptide-like immunoreactivity and catecholamine in neurons of the ventral aminergic pathway of the rat brain. Brain Res. Bull., in press, 1982.

Charlton, C.G., O'Donohue, T.L., Miller, R.L. and Jacobowitz, D.M.: Secretin immunoreactivity in rat and pig brain. Peptides 2, Suppl. 1: 45-49, 1981.

O'Donohue, T.L., Charlton, C.G., Miller, R.L., Boden, G. and Jacobowitz, D.M.: Identification, characterization and distribution of secretin immunoreactivity in rat and pig brain. Proc. Natl. Acad. Sci. USA 78 (8): 5221-5224, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00388-06 LCS |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Neurochemical and Histochemical Studies of Cholinergic Pathways of the Brain | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: David M. Jacobowitz Chief, Histopharmacology Section LCS NIMH | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Histopharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: .7 | PROFESSIONAL: .4 | OTHER: .3 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) There exists a system of dense <u>acetylcholinesterase (ACHE)</u> staining cholinergic <u>cells</u> in the rat forebrain which has been designated as the <u>magnocellular nuclei of the basal forebrain (MnBF)</u> . Because of the diffuseness of the localization of the MnBF, no single lesion of this cell system can reveal the extent of the <u>cholinergic projections</u> throughout the brain. Therefore, a parceling of this chain of cells into segments that are appropriate for stereotaxic lesions seems most feasible. This study was undertaken to elucidate the cholinergic projections emanating from a specific portion of the MnBF, the ventral aspect of the <u>nucleus of the tractus diagonalis (td)</u> . Following lesions <u>choline acetyltransferase (ChAT)</u> was used as the marker to determine cholinergic nerve projection sites. Significant decreases in ChAT activity were observed in the cingulate, frontal and occipital cortices, the hippocampus and dentate gyrus. This study supports the notion that a cholinergic system of cell bodies is contained in the basal forebrain which projects topographically to the neocortex, hippocampus and amygdala. | | |

Project Description:

Objectives: To investigate the cholinergic projection sites of innervation from basal nucleus of the tractus diagonalis (td; diagonal band of Broca).

Methods Employed: (1) Bilateral stereotaxic lesion of the td. (2) Microdissection of brain nuclei. (3) Radioenzymatic assay for choline acetyltransferase (ChAT).

Major Findings: Following a bilateral stereotaxic lesion of the td (9 days), significant decreases in ChAT activity were found in the anterior and posterior cingulate cortex (44% and 20% respectively), the frontal cortex (24%), occipital cortex (74%), dorsal and lateral hippocampus (76% and 65%, respectively) and dentate gyrus (52%).

Significance to Biomedical Research and the Program of the Institute:

This study supports the accumulating evidence that the magnocellular nuclei of the basal forebrain (MNBf) form one continuous system of cholinergic neurons which project topographically to the cortex. The nucleus of the tractus diagonalis has been revealed to be a major cholinergic nucleus innervating many parts of the cortex and basal forebrain structures. This fact, together with the presence of a mesolimbic dopaminergic input (reported by this laboratory last year) from the A10 dopamine cell body area, underlines the potential physiological significance of a dopaminergic/cholinergic interaction at the level of the A10/td system which could have important influences on emotional behavior via connections with the cortex and limbic system. The potential for interaction between dopaminergic and the cholinergic system may prove useful in the future in understanding the neuroanatomical basis for the mechanisms of action of the neuroleptics and other drugs which interact with dopaminergic neurons. For example, the variable anticholinergic properties of neuroleptics are thought to be responsible for the variability in their extrapyramidal effects. Haloperidol, which has little or no anticholinergic activity, causes strong extrapyramidal symptoms. Clozapine, on the other hand, is highly antimuscarinic and is noted for its lack of parkinsonian side effects. Current theories hold that by inhibiting cholinergic transmission as well as dopaminergic transmission in the striatum, the dopaminergic/cholinergic "balance" is maintained by clozapine, whereas it is upset by haloperidol, which blocks only dopamine transmission, in the direction of parkinsonian symptoms. The neurotransmitter anatomical relationships discussed above imply that such a dopaminergic/cholinergic interaction may also be functional with respect to the antipsychotic action of these drugs. Such a functional interaction is supported by the demonstration that the exacerbation of schizophrenic symptoms in patients treated with methylphenidate, a putative dopamine releasing agent, was prevented by treatment with physostigmine, which potentiates the action of acetylcholine (Davis et al., 1977). It must be borne in mind that anticholinergics seem to lessen the extrapyramidal effects of neuroleptic drugs without interfering with their antipsychotic actions. The dopamine/acetylcholine interaction functional in psychosis may therefore be of a different anatomical or pharmacological character from the striatal dopamine/acetylcholine interaction functional in the etiology of extrapyramidal symptoms.

Proposed Course of the Project: Studies of the cholinergic innervation of the brain will continue.

Publications:

Rotter, A. and Jacobowitz, D.M.: Neurochemical identification of cholinergic forebrain projection sites of the nucleus tegmentalis dorsalis lateralis.

Brain Res. Bull. 6: 525-529, 1981.

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|--|---|---|-----|------------------|----------------------------------|-----|------|---------|----------------|----------------|--|-------|--|-------------------|--------------|-----|------|--|-----------------|---------------------|-----|------|--|----------------|-------|-----|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00396-04 LCS | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 to September 30, 1982</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Studies on Substance P as a Neurotransmitter in the Nucleus Tractus Solitarius</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">David Jacobowitz</td> <td style="width: 20%;">Chief, Histopharmacology Section</td> <td style="width: 10%;">LCS</td> <td style="width: 10%;">NIMH</td> </tr> <tr> <td>OTHERS:</td> <td>Cinda J. Helke</td> <td>Pharmacologist</td> <td></td> <td>USUHS</td> </tr> <tr> <td></td> <td>Gloria Feuerstein</td> <td>Guest Worker</td> <td>LCS</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Robert L. Zerbe</td> <td>Senior Staff Fellow</td> <td>LCS</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Irwin J. Kopin</td> <td>Chief</td> <td>LCS</td> <td>NIMH</td> </tr> </table> | | | PI: | David Jacobowitz | Chief, Histopharmacology Section | LCS | NIMH | OTHERS: | Cinda J. Helke | Pharmacologist | | USUHS | | Gloria Feuerstein | Guest Worker | LCS | NIMH | | Robert L. Zerbe | Senior Staff Fellow | LCS | NIMH | | Irwin J. Kopin | Chief | LCS | NIMH |
| PI: | David Jacobowitz | Chief, Histopharmacology Section | LCS | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| OTHERS: | Cinda J. Helke | Pharmacologist | | USUHS | | | | | | | | | | | | | | | | | | | | | | | |
| | Gloria Feuerstein | Guest Worker | LCS | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Robert L. Zerbe | Senior Staff Fellow | LCS | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Irwin J. Kopin | Chief | LCS | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Laboratory of Clinical Science</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION <p style="text-align: center;">Histopharmacology</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <p style="text-align: center;">.8</p> | PROFESSIONAL: <p style="text-align: center;">.5</p> | OTHER: <p style="text-align: center;">.3</p> | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) We have previously reported that a population of afferent nerves from the arterial baroreceptors contain the neuro-peptide substance P (SP). These nerves were traced to the nucleus of the tractus solitarius (NTS) of the medulla oblongata of the hindbrain. Several studies have focused attention on the possible pathogenesis of hypertension by central catecholamine alterations in spontaneously hypertensive (SHR) rats. The present experiment deals with two substrains of the Sabra rat, one of which is genetically predisposed to develop hypertension when fed on a DOCA salt diet (SBH), while the other is hypertension resistant (SBN). In the present experiment SP, catecholamine and vasopressin were studied in the NTS of these rats. Higher concentrations of NE, Epi were observed in the NTS of the SBH strain as compared to the control (SB) or hypertension resistant strain (SBN). The SB was significantly greater than the SBN. SP levels were higher in the SBH and SBN as compared with the SB strain. Vasopressin was higher in SBH and lower in SBN than SB. The significance of a similar directional change for SP in the SBH and SBN strain is unknown. The changes in catecholamines and vasopressin do, however, correlate with the predisposition for hypertension. Whether the changes observed in the catecholamine and peptide systems are causative or adaptive phenomena in the pathogenesis of hypertension susceptibility or resistance awaits further investigation. | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: To determine the concentration of substance P (SP), vasopressin and catecholamine in the nucleus tractus solitarius (NTS) in the Sabra strain of hypertension prone (SBH) and hypertension resistant (SBN) genetically inbred rats that are sensitive and resistant, respectively, to blood pressure elevating procedures such as DOCA-salt treatment or application of a renal arterial clip.

Methods Employed: 1) Genetic substrains of the Sabra rats were obtained from the Hebrew University, Jerusalem. 2) Microdissection techniques for removal of the NTS. 3) Radioimmunoassay of SP and vasopressin. 4) Micro-enzymatic assay for norepinephrine (NE) and epinephrine (Epi).

Major Findings: 1) SP levels in the NTS were significantly higher in the SBH and SBN compared with the control (SB) strain. 2) NE, Epi and vasopressin concentrations were higher in the NTS of the SBH than the SBN and SB strains. The NE and Epi levels in the SB were significantly greater than the SBN.

Significance to Biomedical Research and the Program of the Institute: These are important findings in terms of eventually revealing whether the neuro-peptides or catecholamines studied play a primary or secondary role in the pathogenesis of hypertension susceptibility or resistance.

Proposed Course of the Project: Studies with SP in the brain will continue.

Publications:

Feuerstein, G., Zerbe, R.L., Ben-Ishay, D., Kopin, I.J. and Jacobowitz, D.M.: Catecholamines and vasopressin levels in brain nuclei of the SB, SBH and SBN rats. Brain Res. Bull. 7: 671-676, 1981.

Helke, C.J., DiMicco, J.A., Jacobowitz, D.M. and Kopin, I.J.: Effect of capsaicin administration to neonatal rats on the substance P content of discrete CNS areas. Brain Res. 222: 428-431, 1981.

Helke, C.J., Jacobowitz, D.M. and Thoa, N.B.: Capsaicin and potassium evoked substance P release from the nucleus tractus solitarius and spinal trigeminal nucleus in vitro. Life Sc. 29: 1779-1785, 1981.

Helke, C.J., Goldman, W. and Jacobowitz, D.M.: Demonstration of substance P in aortic nerve afferent fibers by combined use of fluorescent retrograde neuronal labeling and immunocytochemistry. Peptides 1: 359-364, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00397-04 LCS |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies of α-Melanocyte Stimulating Hormone Multi-neurotransmitter Neurons | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Thomas L. O'Donohue OTHERS: David M. Jacobowitz Debra I. Diz Gail E. Handelsmann Y. Peng Loh Victor Hruby | Guest Worker (NIGMS Fellow) Chief, Histopharmacology Section Guest Worker (NIGMS Fellow) Staff Fellow Staff Scientist Professor of Chemistry | LCS NIMH, NIGMS LCS NIMH LCS NIMH, NIGMS LCS NIMH LN NICHD Univ. of Arizona |
| COOPERATING UNITS (if any) Laboratory of Neurobiology, NICHD | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Histopharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 3.2 | PROFESSIONAL: 2.0 | OTHER: 1.2 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Previous studies demonstrated that acetylated and deacetylated forms of α -MSH and β -endorphin exist in the same neurons and pituitary cells in rat and human brain. α -MSH released from the neurons may influence processes of attention, arousal and learning; β -endorphin released from the neurons may influence analgesia. The acetylated form of α -MSH is 2-3 orders of magnitude more potent than deacetylated α -MSH while the acetylated form of β -endorphin is 3-4 fold less potent than β -endorphin. Enzymes which acetylated α -MSH and β -endorphin have been identified in brain and pituitary. Among these is a general acetyltransferase (GAT) present in all organs of the rat. In addition, a specific enzyme capable of acetylating both opiate (β -endorphin) and melanotropic (α -MSH) peptides has been identified, localized to secretory vesicles and named opiomelanotropin acetyltransferase (OMAT). OMAT activity can be induced by physiological manipulation which induces MSH synthesis. Studies of the physiology and pharmacology of α -MSH in the brain have also continued. It was found that α -MSH administration selectively influences visual but not auditory learning. Furthermore, a compound 4-Norleucine, 7-D-Phenylalanine- α -MSH has been synthesized and been found to have identical effects on arousal as α -MSH but opposite effects on learning--suggesting multiple α -MSH receptors in brain. In addition, α -MSH injected into the dorsomedial nucleus of the hypothalamus has been found to cause increases in heart rate. The results of these studies demonstrate that brain α -MSH may be involved in a variety of different physiological functions. | | |

Project Description:

Objectives: Our previous investigations have demonstrated that α -MSH is secreted from neurons in brain which also contain, synthesize and secrete de-acetylated α -MSH, β -endorphin and acetylated β -endorphin. The objectives of these studies were 1) to determine the mechanism of acetylation of α -MSH and β -endorphin; 2) to study regulation of the α -MSH and β -endorphin acetylating enzymes; 3) to determine if there are specific sensory modalities influenced by pharmacological administration of α -MSH; 4) to determine if different behavioral actions of α -MSH can be pharmacologically dissected; 5) to determine the influence of brain α -MSH on blood pressure and heart rates.

Methods Employed: 1) Develop assays for α -MSH and β -endorphin acetyltransferases; 2) α -MSH and β -endorphin radioimmunoassay; 3) Subcellular fractionation; 4) Testing of attentional and arousal processes after MSH administration; 5) High pressure liquid chromatography; 6) Solid-phase peptide synthesis; 7) Measurement of blood pressure and heart rate during and after intrahypothalamic injections of α -MSH.

Major Findings: 1) Identification of a specific enzyme opiomelanotropin acetyltransferase (OMAT) which can specifically acetylate α -MSH and β -endorphin; 2) OMAT is specifically localized to cells which synthesize α -MSH and β -endorphin; 3) OMAT is specifically localized to α -MSH and β -endorphin secretory vesicles; 4) OMAT has a pH optima of 5.5-6.0 -- identical to the internal pH of the vesicles; 5) OMAT activity can be induced by manipulations which induce α -MSH biosynthesis; 6) Identification of a general acetyltransferase (GAT) present in all tissues which is capable of acetylating α -MSH; 7) Pharmacological administration of α -MSH specifically affects visual but not auditory discrimination; 8) 4-Norleucine, 7-D-Phenylalanine- α -MSH influences arousal processes identically to α -MSH but has the opposite effect on learning processes, suggesting multiple α -MSH receptors in brain; 9) Injection of α -MSH into the dorsomedial hypothalamic nucleus of the rat induced a 13% increase in heart rate and a slight increase in blood pressure. In contrast, injections of α -MSH into the preoptic medial nucleus, anterior, paraventricular and posterior hypothalamic nuclei, lateral ventricle or femoral vein were without effect on blood pressure and heart rate.

Significance to Biomedical Research and the Program of the Institute: The α -MSH containing neuron provides the first model of a multi-neuro-transmitter neuron. It is hypothesized that many neurons may actually be multi-neurotransmitter neurons with the other co-transmitters yet to be discovered. The study of the pre- and post-synaptic regulation and action of the α -MSH-containing neuron should provide a first insight into the basic functioning of multi-neurotransmitter and multi-hormonal cells.

Proposed Course of Project: Further studies to understand pre- and post-synaptic regulation of the multi-neurotransmitter neuron are in progress.

Publications:

O'Donohue, T.L. and Chappell, M.C.: Distribution of an enzyme which acetylates α -melanocyte stimulating hormone in rat brain and pituitary gland and effects of arcuate nucleus lesions. Peptides 3: 69-75, 1982.

O'Donohue, T.L., Handelsmann, G.E., Miller, R.L. and Jacobowitz, D.M.: N-acetylation regulates the behavioral activity of α -melanotropin in a multi-neurotransmitter neuron. Science 215: 1125-1127, 1982.

O'Donohue, T.L., Handelsmann, G.E., Chaconas, T., Miller, R.L. and Jacobowitz, D.M.: Evidence that N-acetylation regulates the behavioral activity of α -MSH in the rat and human central nervous system. Peptides 2: 333-344, 1981.

O'Donohue, T.L.: Identification of endorphin acetylating enzyme (EAE) in rat brain and pituitary gland. Journal of Biological Chemistry, in press, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00401-17 LCS |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Mechanisms Regulating Formation, Release, Disposition, Metabolism, and Actions of Norepinephrine. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Irwin J. Kopin OTHER: Zofia Zukowska-Grojec Mohamed Bayorh Chuang C. Chiueh | Chief, LCS Visiting Fellow Visiting Fellow Sr. Staff Fellow | LCS NIMH LCS NIMH LCS NIMH LCS NIMH |
| COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIADD, NIH | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Medicine | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 2.0 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this project are to elucidate biochemical mechanisms controlling the <u>synthesis, storage, release, action and termination of action of norepinephrine</u> in the adrenergic neurones and how to assess these in the intact animal. This year attention has focused <u>6-fluoronorepinephrine</u> as a substitute transmitter for norepinephrine and on evaluation of functional aspects of α -adrenoceptor function, <u>intrajunctional (α_1-) and extrajunctional (α_2-) locations of α-adrenoceptors</u> have been further defined. A method has been developed to <u>estimate the concentration of norepinephrine at α_1-adrenoceptors</u> which regulate blood pressure responses in pithed rats and is being applied to studies of disposition of released norepinephrine and regulation of α_1 -adrenoceptors. | | |

PROJECT DESCRIPTION:

The basis for position emission tomography (PET) scanning of brain and other tissues is the selective uptake and retention at specific sites of a substance labelled with a radioactive atom which emits a positron as it decays. The radioactive element which is most frequently used is ^{18}F . Studies have been initiated (Drs. Chiueh, Daly, and Kopin) on the disposition and metabolism of ^{18}F -labelled catecholamines and their precursor, 6-fluorodopa, to examine the feasibility of studying catecholaminergic neuronal function. Techniques for separation and assay in tissues and fluids of non-radioactive fluorinated catecholamines and some of their metabolites have been developed. It has been shown that 6-fluorodopamine (6-F DA) is a false transmitter precursor which is taken up selectively by sympathetic nerves and converted to 6-F-norepinephrine (6-F-NE). The 6-F-NE is stored in the vesicles of sympathetic nerves and released during stimulation. This compound differs somewhat from norepinephrine in its specificity for receptors and may be a useful specific therapeutic application as well as a useful drug for PET scans or as a pharmacological tool.

Pithed rats are useful for studying changes in release of norepinephrine or responses to sympathetic stimulation because compensatory reflexes mediated by the central nervous system are absent and because sympathetic outflow from the spinal cord can be controlled rigorously. Rats made hypertensive by treatment with DOCA and salt or by renal compression have increased sensitivity to high doses of administered norepinephrine, but threshold responses occurred at similar doses (Drs. DiMicco, Feuerstein and Kopin). Although the pressor effects of sympathetic stimulation were greater in hypertensive than in control pithed rats, the hypertensive rats appear to have released less norepinephrine. This could be due to inhibition of NE uptake with enhanced actions at presynaptic release-inhibiting receptors as well as increased sensitivity of the α -receptors. This distinction can now be studied by methods recently developed (see below) to measure levels of NE at the neuroeffector junction.

Phencyclidine (PCP) enhances cardiovascular responses and NE release in pithed rats as well as in intact animals. (Drs. Bayorh and Kopin). The drug does not interfere with uptake of administered NE, but does enhance stimulation-induced increments in plasma NE. A similar effect in awake rats is attributable to both peripheral actions on NE release as well as central nervous system effects on sympathetic outflow.

As a result of research performed in this laboratory in previous years, we have proposed that α_1 -adrenoceptors are located mainly within neuroeffector junctions or synapses whereas α_2 -adrenoceptors are primarily extrajunctional. This conclusion was based on results obtained with relatively non-specific α_1 - and α_2 -adrenoceptor blocking agents. We (Drs. Zukowska-Grojec, Bayorh, and Kopin) have now extended these observations to include more specific α_1 - and α_2 -adrenoceptor blocking agents. The effects of α_1 - and α_2 -adrenoceptor blocking agents on the pressor response to sympathetic stimulation are additive, indicating that administered norepinephrine reaches both type receptors. Desmethyl-imipramine and cocaine, which block reuptake of norepinephrine into sympathetic

nerves, do not greatly potentiate the pressor response to stimulation of the sympathetic outflow from the spinal cord of pithed rats, but do prolong and potentiate the effects of administered norepinephrine. This difference is in part due to the effects of presynaptic α_2 -adrenoceptors which modulate feedback control by norepinephrine of its own release. When uptake is inhibited a greater fraction of the released norepinephrine reaches extrajunctional (α_2) receptors. The enhanced effects at presynaptic (α_2) adrenoceptors, decreases the amounts of norepinephrine released so that the net action of α_1 -adrenoceptors in the junction and the overflow of norepinephrine to α_2 -adrenoceptors is not greatly altered. This interpretation is supported by the effects of yohimbine (an α_2 -adrenoceptor blocking agent) and prazosin (α_1 -adrenoceptor blocking agent) in pithed rats pretreated with DMI. Inhibition of uptake by DMI reverses more effectively the inhibition by yohimbine than by prazosin of stimulation-induced pressor responses. This is attributed to the reversal of the presynaptically (α_2 -adrenoceptor) mediated inhibition of norepinephrine release.

The effects of inhibition of uptake by DMI on the pressor effects of administered norepinephrine after treatment of pithed rats with yohimbine and prazosin also support the view that α_2 -adrenoceptors are extrajunctional and α_1 -are intrajunctional. After inhibition of α_2 -adrenoceptors (yohimbine), DMI potentiates by 10-fold pressor effects of administered norepinephrine at the remaining α_1 -receptors. This is due to a combination of higher plasma levels of norepinephrine (because uptake is inhibited everywhere) and greater accessibility of plasma NE to the α_1 - (intrajunctional) receptors. Potentiation by DMI of the pressor responses after inhibition of α_1 -adrenoceptors is only about 3.5-fold. Since the remaining (α_2 -) receptors are extrajunctional, the potentiation is mainly a result of higher plasma NE levels. The difference in potentiation by DMI of actions of administered NE at α_1 -(intrajunctional) and α_2 -(extrajunctional) receptors may be explained by their location in relation to the site of NE uptake which is inhibited by DMI.

It can be shown that the gradient of concentration of infused NE between the plasma and the α_1 -intrajunctional receptor results in a fall in concentration which is similar but in opposite directions to that for NE released during sympathetic nerve stimulation. By comparing in pithed rats the blood pressure response-plasma norepinephrine level relationships during infusion and during constant sympathetic stimulation the concentration of NE at the intrajunctional α_1 -adrenoceptor can be deduced. This method can be used to distinguish changes in accessibility of NE to receptors from changes in sensitivity of α_1 -adrenoceptors and may be able to be adapted for use in humans to study autonomic nervous system disorders or effects of therapeutic agents on receptors.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

Norepinephrine is the neurotransmitter released for sympathetic nerves and some neurones in brain. Understanding its formation, disposition, metabolism and action are fundamental to defining its role in disease states and during drug action.

PROPOSED COURSE:

Continued study of processes and their regulation in experimental animals.

PUBLICATIONS

Kopin, I.J.: False transmitters revisited: Their role in the hypotensive action of MAO inhibitors. In Kamijo, K., Usdin, E. and Nagatsu, T. (Eds.): Monoamine Oxidase. Basic and Clinical Frontiers. Amsterdam-Oxford-Princeton, Excerpta Medica 1981, pp. 321-327.

Kopin, I.J.: The evolving views of the metabolic fate of norepinephrine. Endocrinologia Experimentalis. In press.

Yamaguchi, I., Torda, T., Hirata, F., and Kopin, I.J.: Adrenoceptor desensitization after immobilization stress or repeated injection of isoproterenol. Am. J. Physiol. 240: (Heart Circ. Physiol. 9:) H691-H696, 1981.

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|--|---|---|--------------------|------------|----------|------------------------|--------------|----------|----------------|--------------|----------|----------------|--------------|----------|------------------|--------------|----------|------------------|--------------|----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00402-10 LCS | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) CNS Regulation of Autonomic and Endocrine Function | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Irwin J. Kopin</td> <td style="width: 33%;">Chief, LCS</td> <td style="width: 33%;">LCS NIMH</td> </tr> <tr> <td>OTHER: Anthony Zavadil</td> <td>Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td>Charles Saller</td> <td>Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td>David Lozovsky</td> <td>Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td>Giora Feuerstein</td> <td>Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td>Andreas Pfeiffer</td> <td>Guest Worker</td> <td>LCS NIMH</td> </tr> </table> | | | PI: Irwin J. Kopin | Chief, LCS | LCS NIMH | OTHER: Anthony Zavadil | Guest Worker | LCS NIMH | Charles Saller | Guest Worker | LCS NIMH | David Lozovsky | Guest Worker | LCS NIMH | Giora Feuerstein | Guest Worker | LCS NIMH | Andreas Pfeiffer | Guest Worker | LCS NIMH |
| PI: Irwin J. Kopin | Chief, LCS | LCS NIMH | | | | | | | | | | | | | | | | | | |
| OTHER: Anthony Zavadil | Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | |
| Charles Saller | Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | |
| David Lozovsky | Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | |
| Giora Feuerstein | Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | |
| Andreas Pfeiffer | Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Sections on Histopharmacology and Pharmacology Dept. of Neurobiology, USUHS | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Medicine | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 2.5 | OTHER: 0 | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to define the regions of brain, the neuronal pathways, and the neurotransmitter systems which control endocrine, metabolic and autonomic responses to internal stimuli (e.g., blood glucose, O ₂ -tension, hormonal state, body temperature, etc.) and to various types of stress (immobilization, foot-shock, hemorrhage, etc.) and the effects of drugs on these systems. The possible role of prostaglandins in control of autonomic function has been examined by studying the effects on cardiovascular parameters of various prostaglandins injected into specific brain areas. Similar studies using opiate peptides which selectively act at mu or delta receptors were completed. The role of vasopressin as a neurotransmitter affecting cardiovascular control mechanisms was studied in Sabra hypertension-prone and-resistant strains. Previous observations on the interaction of glucose and dopaminergic neurones have been extended to studies of dopaminergic receptors. | | | | | | | | | | | | | | | | | | | | |

Integration by the central nervous system of information from baro- and chemoreceptors and other sensors of the internal environment results in reflex modulation of respiratory, metabolic, endocrine and cardiovascular function. Using surgical techniques for lesioning or microinjection of appropriate pharmacological agents, specific neuronal pathways may be interrupted or stimulated. Microdissection and microassay of putative neurotransmitters aid identification of specific neuronal systems.

A series of studies on the cardiovascular and metabolic effects of prostaglandins injected directly into the cerebral ventricles or specific brain nuclei of anesthetized rats have been completed (Drs. Feuerstein, Helke, Adelberg, Kopin and Jacobowitz). PGE₂ injected into the lateral cerebral ventricle or directly into the dorsomedial or posterior hypothalamic nuclei evokes pressor responses attended by increases in plasma norepinephrine but not epinephrine. Intracisternal injections of PGI₂ elicit only mild responses and only at doses which are sufficiently large to have systemic effects when injected intravenously; when injected into the dorsomedial nucleus, PGI₂ may have a selective effect on heart rate. PGF₂ injections in the paraventricular, dorsomedial, or posterior hypothalamic nuclei evoke marked increases in heart rate and blood pressure attended by increases in plasma catecholamines. Higher doses of PGF₂ injected into the cerebral ventricles also elicit increases in heart rate, blood pressure, respiration and rectal temperature. Vagotomy has little effect on the responses, but hexamethonium blocks the pressor and temperature responses but not the heart rate or respiratory responses. Removal of the kidneys abolishes the residual respiratory, pressor, and temperature responses in hexamethonium-treated rats, indicating that renin-angiotensin might be involved.

The role of opiate peptides in the regulation of cardiovascular responses to hypotension and control of blood pressure is the subject of another series of studies. Naloxone, an opiate antagonist, increases the rate of recovery of the lowered blood pressure attending severe hemorrhage, but has no significant effect on plasma catecholamine levels, indicating that the effect of naloxone is mediated by other pressor mechanisms (Drs. Feuerstein, Chiueh and Kopin). By locally injecting selective opioid receptor agonists into specific areas of brain, the possible roles of different types of receptors in mediating regulation of the autonomic nervous system can be assessed (Drs. Pfeiffer, Feuerstein, Faden and Kopin). Injection of a highly μ -receptor agonist enkephaline analogue (D-Ala², Mc Phe⁴ glycol, DAGO) or a less selective delta-agonist (D-Ala² D-Lve, DADL) evokes increases in blood pressure, heart rate, and catecholamine levels in plasma. These effects are prevented by naloxone. At higher doses the effect on heart rate is diminished, but subsequent treatment with naloxone evokes an almost instantaneous increase in heart rate which can also be obtained with a peripheral muscarinic blocking agent, atropine methyl nitrate. The effects of naloxone and methylatropine are not additive, indicating that the drugs have a similar effect in removing vagal influences, thereby unmasking a high level of sympatho-adrenal activity. The greater potency of the μ agonist is consistent with the high density of μ receptors in the anterior hypothalamus. Chronic treatment of spontaneously hypertensive (SHR) rats with naloxone administered by continuous infusion from osmotic minipumps for 4 weeks, produces striking increases (80-100%) in the number of

opiate binding sites in the anterior hypothalamus and brain stem. (Drs. Pfeiffer, Feuerstein, Faden and Kopin). These animals are more sensitive to cardiac depressant, but not to respiratory depressant effects of morphine. The increase in blood pressure is unaffected by the treatment although body weights are less in treated than untreated SHR rats. These results indicate that there is a functional relationship between cardiovascular depressant effects and inducible opiate receptors, and that these differ from receptors mediating respiratory depression.

Vasopressin is an antidiuretic peptide secreted from the posterior pituitary, but is present in many areas of the brain. It is secreted into the circulation in response to various stresses as well as to an increase in plasma osmotic pressure. Osmotic stimulation has little or no effect on the low levels of vasopressin outside the hypothalamo-pituitary stalk regions, suggesting that the endocrine and neurotransmitter functions of vasopressin are separately controlled (Drs. Zerbe, Palkovits and Kopin). The levels of vasopressin in the caudal portion of the nucleus tractus solitarius (NTS) are higher in hypertension-prone Sabra rats and lower in the resistant strain than in unselected Sabra rats. Catecholamine levels in the NTS and locus coeruleus are similarly affected. These changes may be primary or secondary to the differences in blood pressure of these rats.

Previous observations on the effects of blood glucose on dopaminergic mechanisms in brain have been extended to examine dopamine receptors. Glucose was previously shown to depress dopaminergic neurone firing rates; this should decrease dopamine stimulation of its receptors. Decreased transmitter release frequently results in an increase in the density of the receptors for the transmitter. Consistent with such an effect, dopamine receptors were found to be increased in rats made hypoglycemic by induction of diabetes (Drs. Lazovsky, Saller, and Kopin). This is reversed by insulin treatment and prevented by chronic treatment with lithium. The decrease in dopamine release attending glucose administration potentiates the cataleptic effects of the dopamine antagonist, haloperidol, indicating a functional consequence of the neurophysiologic effect of glucose (Drs. Saller and Kopin). Prolactin secretion is thought to be inhibited by dopaminergic stimulation. Glucose administration increases and fasting decreases prolactin levels (Drs Saller, Zerbe and Kopin) consistent with the effects of glucose on dopaminergic neurone activity. Phencyclidine (PCP) decreases prolactin levels in plasma, presumably by activation of dopamine receptors because this effect is blocked by haloperidol (Drs. Saller, Zerbe, Bayorh and Kopin). Naloxane has a very small effect in reversing this effect of PCP indicating that the effect is not mediated by opiate mechanisms.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

The brain regulates a variety of important metabolic processes. The mechanism for this regulation may be abnormal in disease states or during drug action. Fundamental information on sites of brain, particular neurotransmitters, and pathways for this regulation is required to understand mechanisms of disorders of these functions in neuropsychiatric diseases and during drug therapies.

PROPOSED COURSE:

Continued studies on areas of brain required for metabolism effects, cardiovascular responses, etc. and evaluation of the roles of various peptides and other neurotransmitters, and the effects of drugs which affect neurotransmitter function.

PUBLICATIONS:

Feuerstein, G., Adelberg, S.A., Kopin, I.J., and Jacobowitz, D.M.: Hypothalamic sites for cardiovascular and sympathetic modulation by prostaglandin E₂. Brain Res. 231: 335-342, 1982.

Feuerstein, G., Adelberg, S.A., Kopin, I.J. and Jacobowitz, D.M.: Central cardiovascular effects of prostacyclin. Neuropharmacology 20: 1085-1090, 1981.

Feuerstein, G., Chiueh, C.C., and Kopin, I.J.: Effect of naloxone on the cardiovascular and sympathetic response to hypovolemic hypotension in the rat. Eur. J. Pharmacol. 75: 65-69, 1981.

Feuerstein, G., Helke, C.J., Jacobowitz, D.M., and Kopin, I.J.: Mechanisms involved in central effects of PGF₂ alpha. Am. J. Physiol. In press.

Kopin, I.J.: Mode of action of antidepressants and central stimulants. In van Praag, H.M., Lader, M.H., Rafaelsen, O.J., and Sachar, E.J. (Eds.): Handbook of Biological Psychiatry - part IV Brain Mechanisms and Abnormal Behavior. New York, Marcel Dekker, 1981, pp. 741-766.

Lozovsky, D., Saller, C., and Kopin, I.J.: Dopamine receptor binding is increased in diabetic rats. Science 214: 1031-1032, 1981.

Kopin, I.J.: Neurotransmitters and the Lesch-Nyhan syndrome. N. Eng. J. Med. 305 1148-1150, 1981.

Saller, C.F., and Kopin, I.J.: Glucose potentiates haloperidol induced catalepsy. Life Sci. 29(22): 2337-2341, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00403-09 LCS | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Biochemical Indices of Autonomic Function in Humans | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: Irwin J. Kopin</td> <td style="width: 40%;">Chief, LCS</td> <td style="width: 30%;">LCS NIMH</td> </tr> <tr> <td>OTHER: Virginia Wiese</td> <td>Chemist</td> <td>LCS NIMH</td> </tr> <tr> <td>Young Kim</td> <td>Anesthesiologist</td> <td>CC NIH</td> </tr> <tr> <td>Edna K. Gordon</td> <td>Chemist</td> <td>LCS NIMH</td> </tr> <tr> <td>Michael H. Ebert</td> <td>Chief, Section on Experimental Therapeutics</td> <td>LCS NIMH</td> </tr> <tr> <td>Jerry A. Oliver</td> <td>Chemist</td> <td>LCS NIMH</td> </tr> <tr> <td>Ronald Polinsky</td> <td>Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td>Ib Oddershede</td> <td>Visiting Fellow</td> <td>LCS NIMH</td> </tr> </table> | | | PI: Irwin J. Kopin | Chief, LCS | LCS NIMH | OTHER: Virginia Wiese | Chemist | LCS NIMH | Young Kim | Anesthesiologist | CC NIH | Edna K. Gordon | Chemist | LCS NIMH | Michael H. Ebert | Chief, Section on Experimental Therapeutics | LCS NIMH | Jerry A. Oliver | Chemist | LCS NIMH | Ronald Polinsky | Guest Worker | LCS NIMH | Ib Oddershede | Visiting Fellow | LCS NIMH |
| PI: Irwin J. Kopin | Chief, LCS | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| OTHER: Virginia Wiese | Chemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| Young Kim | Anesthesiologist | CC NIH | | | | | | | | | | | | | | | | | | | | | | | | |
| Edna K. Gordon | Chemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| Michael H. Ebert | Chief, Section on Experimental Therapeutics | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| Jerry A. Oliver | Chemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| Ronald Polinsky | Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| Ib Oddershede | Visiting Fellow | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS: Department of Medicine, Mt. Sinai Hospital, New York, N.Y.; Department of Anesthesiology, NIH; Office of the <u>Director, NHLBI</u> | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Medicine | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 3.0 | OTHER: 1.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The levels and turnover rates of <u>norepinephrine</u> , <u>epinephrine</u> , and <u>dopamine</u> and their various <u>metabolites in body fluids</u> reflect to different degrees the level of functions of the aminergic neurones from which the amines are released. Peripheral sympathetic responsivity is reflected in plasma levels of norepinephrine but care must be taken to consider removal rates of the catecholamine. Urinary metabolites of the catecholamines provide a useful means for assessing their overall synthesis in the body while the relative proportion of <u>normetanephrine</u> appears to be related to functional activity. Other indirect indices, based on stimulation of secretion of other compounds (melatonin, pancreatic polypeptide), are also valuable adjuncts in assessing <u>autonomic activity</u> . This project now includes Z01-MH 00275 which it has grown to overlap. | | | | | | | | | | | | | | | | | | | | | | | | | | |

PROJECT DESCRIPTION:

The autonomic nervous system is regulated by the central nervous system which orchestrates via reflex and hormonal mechanisms a variety of important physiological functions. Plasma levels of catecholamines, renin, cortisol, vasopressin, and other peptides reflect activities of these mechanisms. Plasma norepinephrine reflects acute changes in sympathetic nerve activity and has been a focus for more intensive studies.

Plasma norepinephrine levels are maintained by overflow from the norepinephrine released at sympathetic nerve endings. The levels are determined by the rate of removal of the catecholamine from plasma as well as the proportion of the released transmitter which is removed by reuptake or metabolism before reaching the circulation. It is therefore important to study uptake as well as rates of removal of the catecholamine from the circulation. A method developed for assay of catecholamines by high performance liquid chromatography and electrochemical detection (HPLC-ED) has been adopted for assaying simultaneously endogenous plasma norepinephrine and collection of the separated radioactively labelled norepinephrine and isoproterenol for assay of tritium or carbon contents (Drs. Goldstein, Feuerstein, Izzo, Kopin and Kaiser). This method has been applied to studies in normal subjects and in patients with hypertension or with orthostatic hypotension (Drs. Goldstein, Horowitz, Keiser, Polinsky and Kopin). Hypertensive patients with elevated levels of plasma norepinephrine have similar clearance rates for both ^3H - ℓ -norepinephrine and ^{14}C - d -norepinephrine as do normal subjects. The clearance rates for d - and ℓ -isomers appear to be identical, indicating lack of stereospecificity for mechanisms of removal of norepinephrine from the circulation. ^3H -isoproterenol is not taken up into sympathetic nerves and is cleared more slowly than is norepinephrine. This produces a difference in the ratio of ^3H -isoproterenol to ^3H - ℓ -norepinephrine, particularly after cessation of the infusion. The difference is abolished by treatment with a drug, desipramine, which blocks uptake of norepinephrine into nerve endings. There is no apparent difference in the uptake mechanism in hypertensive and normotensive subjects. This indicates that the increased levels of NE in hypertensive patients is due to enhanced release rather than decreased removal of NE after its release.

Levels of norepinephrine in venous plasma are the net effects of uptake of NE from arterial plasma and release of NE during passage through the tissues. Arterial levels of NE are related in the same manner to levels of NE in mixed venous blood because of NE uptake and release during passage of blood through the lungs. Measurements of plasma NE levels in peripheral (antecubital vein) arterial central venous and coronary sinus blood have been used to assess regional changes in net release of NE during cardiac bypass operations (Drs. Kim, Jones, Hanowell, Koch, Lees, Mrs. Weise, and Dr. Kopin). After induction of anesthesia, plasma NE levels everywhere decrease, but increase when the chest is opened. After completion of the bypass, the peripheral venous blood contains highest levels of NE and the heart begins to take up, rather than release, NE.

In a collaborative study (Drs. Bybee, Wiesen, Aronin, Krieger, Frohman and Kopin) we have been unable to confirm that bromocryptine, a dopamine agonist, lowers plasma norepinephrine levels.

On the basis of previous studies in our laboratory, patients with orthostatic hypotension are thought to suffer from deficits in activation of the sympathetic outflow from the central nervous system or from direct involvement of the peripheral sympathetic nervous system. Patients with isolated orthostatic hypotension (IOH) have only symptoms of autonomic deficits - e.g., hypotension, impotence, sweating disorders, etc., whereas those with central nervous system disorder (Multiple System Atrophy, MSA) have symptoms referable to brain - e.g., tremor, incoordination, rigidity, etc., as well as the deficits in autonomic function. Patients with IOH excrete abnormally low amounts of all major catecholamine metabolites - 3-methoxy-4-hydroxymandelic acid (VMA), 3-methoxy-4-hydroxyphenyl glycol (MHPG), and normetanephrine. The proportion of each metabolite remains normal. This is attributed to loss of sympathetic nerves but normal functioning of the relatively few remaining neurones. The pattern of urinary NE metabolites is different in patients with MSA. These patients excrete equally low amounts of normetanephrine but only slightly less than normal amounts of VMA and MHPG. The disproportionate decrease in normetanephrine is explained by the inability of these patients to activate appropriately release of norepinephrine which can still be formed and metabolized intraneuronally.

Many years ago we (Dr. Kopin and Mrs. Gordon, 1961) had shown that released norepinephrine is preferentially O-methylated and a proportionately large fraction of the amine is excreted as normetanephrine than of norepinephrine metabolized in sympathetic neurones, before it is released. In patients with MSA synthesis of NE in the sympathetic nerves proceeds at a near-normal rate, but the amine is metabolized (by deamination) before being released because the nerves are not activated. There is, therefore, a more striking decrease in normetanephrine than in total norepinephrine metabolites (Drs. Kopin, Ebert, Ms. Gordon, Dr. Oddershede, Mr. Oliver and Dr. Polinsky). Studies in patients with various forms of anxiety suggest that activation of sympathetic discharge results in disproportionate increases in normetanephrine and/or metanephrine excretion.

Patients with IOH and MSA have been shown to have deficient adrenal medullary responses to hypoglycemia (Drs. Polinsky, Kopin, Ebert, Mrs. Weise, and Dr. Recant) but are still able to respond with increases in glucagon to correct the blood glucose. Patients with IOH secrete markedly decreased amounts of the metabolite of melatonin, 6-hydroxymelatonin, primarily because of failure to activate the usual evening rise in secretion of the pineal hormone, but when they do secrete the reduced amounts of melatonin, it is secreted at night. This differs in patients with MSA, who secrete the hormone inappropriately (during the day as well as the night) although the secretion rates are usually low (Drs. Tetsuo, Polinsky, Markey and Kopin). There is also evidence that vagally-mediated hypoglycemia-induced secretion of pancreatic polypeptide is deficient in patients with orthostatic hypotension, indicating involvement of the parasympathetic as well as sympathetic nervous systems (Drs. Polinsky, Taylor, Mrs. Weise and Dr. Kopin).

Deuterium-labelled homovanillic acid ingested intravenously mixes rapidly with the endogenous compound and provides a useful means of studying the pool size, turnover, and synthesis rates of the dopamine metabolite (Drs. Elchisak, Polinsky, Ebert, Modlin, and Kopin). The biological half-life of HVA in monkeys ranges from 30 min. to 100 min. Only about 50% of the administered dose is excreted in urine, the remainder being metabolized or excreted in bile.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

Indices of autonomic activity in humans are sought to find means of evaluating autonomic activity abnormalities in patients with various neurologic and psychiatric disorders and to study the affects of drugs.

PROPOSED COURSE:

Continue to study the mechanisms involved in regulation of catecholamine metabolism and disposition in humans with various neuropsychiatric disorders using excretion rates, blood and CSF levels, and isotopic studies of kinetics of formation of biogenic amine metabolites in humans.

PUBLICATIONS:

Bybee, D.E., Wiesen, M., Aronin, N., Krieger, D.T., Frohman, L.A. and Kopin, I.J.: Failure of bromocriptine to lower plasma catecholamines in normal men and women. J. Clin. Endocrinol. Metab. In press.

Elchisak, M.A., Polinsky, R.J., Ebert, M.H. and Kopin, I.J.: Kinetics of homovanillic acid and determination of its production rate in humans. J. Neurochem. 38: 380-385, 1982.

Goldstein, D.S., Feuerstein, G., Izzo, J.L., Kopin, I.J. and Keiser, H.R.: Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man. Life Sci. 28: 467-475, 1981.

Kim, Y.D., Jones, M., Hanowell, S.T., Koch, J.P., Lees, D.E., Weise, V. and Kopin, I.J.: Changes in peripheral vascular and cardiac sympathetic activity before and after coronary artery bypass operations: Interrelationships with hemodynamic alterations. Am. Heart J. 102: 972-979, 1981.

Kopin, I.J.: Plasma catecholamines as an index of a neuroendocrine response. Psychoneuroendocrine dysfunction in psychiatric and neurological illness. In press.

Kopin, I.J.: Plasma levels of catecholamines and dopamine-beta-hydroxylase. In U. Trendelenburg and N. Weiner (Eds.): Catecholamines II - Handbook of Experimental Pharmacology. Berlin-Heidelberg, Springer-Verlag, In press.

Polinsky, R.J., Kopin, I.J., Ebert, M.H., Weise, V. and Recant, L.: Hormonal responses to hypoglycemia in orthostatic hypotension patients with adrenergic insufficiency. Life Sci. 29: 417-425, 1981.

Polinsky, R.J., Taylor, I.L., Weise, V., and Kopin, I.J.: Pancreatic polypeptide responses to hypoglycemia in chronic autonomic failure. J. Clin. Endocrin. Med. 54: 5401-5408, 1982.

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|--|--|---|----------------------|----------------|------------|----------|--------|---------------------|-----------------|----------|--|-----------------|--------------|----------|--|-------------------|---------|----------|--|---------------|-----------------|----------|--|-----------------|--------------------------------|----------------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00404-11 LCS | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Immunological Localization of GAD and CSD in Neurones and Other Cells | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Irwin J. Kopin</td> <td style="width: 20%;">Chief, LCS</td> <td style="width: 20%;">LCS NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Donald E. Schmechel</td> <td>Medical Officer</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Wolfgang Oertel</td> <td>Guest Worker</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Virginia K. Weise</td> <td>Chemist</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Marcel Tappaz</td> <td>Visiting Fellow</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Enrico Mugnaini</td> <td>Chief, Lab. of Neuromorphology</td> <td>State U. Connecticut</td> </tr> </table> | | | PI: | Irwin J. Kopin | Chief, LCS | LCS NIMH | OTHER: | Donald E. Schmechel | Medical Officer | LCS NIMH | | Wolfgang Oertel | Guest Worker | LCS NIMH | | Virginia K. Weise | Chemist | LCS NIMH | | Marcel Tappaz | Visiting Fellow | LCS NIMH | | Enrico Mugnaini | Chief, Lab. of Neuromorphology | State U. Connecticut |
| PI: | Irwin J. Kopin | Chief, LCS | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| OTHER: | Donald E. Schmechel | Medical Officer | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Wolfgang Oertel | Guest Worker | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Virginia K. Weise | Chemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Marcel Tappaz | Visiting Fellow | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Enrico Mugnaini | Chief, Lab. of Neuromorphology | State U. Connecticut | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) State University of Connecticut, Storrs, Connecticut | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Medicine | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0 | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Immunohistological methods are being used to define the distribution in the nervous system and other tissues of cells (neurones, glia, etc.) which contain specific enzymes associated with particular functions (e.g., production of specific neurotransmitters, hormones, etc.). The ontogeny of the specific proteins and their role in <u>growth, differentiation</u> and <u>regenerative processes</u> are being examined using these techniques. | | | | | | | | | | | | | | | | | | | | | | | | | | |

PROJECT DESCRIPTION:

Histological demonstration of the presence in a neurone or its nerve endings of an enzyme required for the biosynthesis of a neurotransmitter is highly suggestive and generally accepted evidence that the neurone secretes that neurotransmitter. Thus localization by immunohistochemical methods of glutamic acid decarboxylase (GAD) in a particular neurone indicates that α -aminobutyric acid (GABA) is the neurotransmitter released from the neurone. Antibodies to glutamic acid decarboxylase (GAD) which were previously made and characterized in our laboratory were used to study the enzyme in brain. Using immunocytochemical techniques on the light- and electron-microscopic level, GAD-immunoreactivity was localized specifically in different brain areas, including the cerebellum, olfactory bulb, cerebral cortex, basal ganglia and substantia nigra in rat brain. In order to reveal GABAergic pathways, GAD-immunoreactivity was also investigated in specific brain areas after injection of neurotoxic agents and selective neurosurgery. Studies on the functional anatomy of the GABAergic system of the rat and characterization of GABAergic components in different tissue culture systems are in progress. The GAD activity is present in the same cultured cells which take up and store ^3H -GABA. The high titre and excellent specificity of the GAD antibody have led many investigators throughout the world to seek (and obtain) use of our antibody for their studies.

Glutamic acid decarboxylase was compared with the enzyme activity of cysteine sulfinic acid decarboxylase (CSD), the proposed biosynthetic enzyme for hypotaurine and taurine. CSD activity was separated in two enzyme activities, termed CSD I and CSD II. CSD I was found to be extremely similar to liver CSD, but CSD II was indistinguishable by biochemical and immunological techniques from GAD. Antibodies to CSD I have been raised in sheep and have been used to definitively distinguish between the two forms of CSD.

In the pituitary, GAD immunoreactivity was found in the neural and intermediate lobes. This GAD disappeared with stalk section, but CSD I activity remained, demonstrating the difference in location as well as specificity of the enzyme activities.

A double-staining method to demonstrate simultaneously the coincident localization of GAD and somatostatin has been developed and these two substances shown to be present in the same cells of the nucleus reticularis of the thalamus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

Knowledge of the location and axonal distribution of neurotransmitters is required to understand mechanisms of brain function.

PROPOSED COURSE:

Except for special purposes of collaboration this project will be terminated during the next fiscal year.

PUBLICATIONS:

Oertel, W.H., Mugnaini, E., Tappaz, M.L., Weise, V.K., Dahl, A.L., Schmechel, D. E. and Kopin, I.J.: Central GABAergic innervation of neurointermediate pituitary lobe: Biochemical and immunocytochemical study in the rat. Neurobiology 79: 675-679, 1982.

Oertel, W.H., Schmechel, D.E., Mugnaini, E., Tappaz, M.L. and Kopin, I.J.: Immunocytochemical localization of glutamate decarboxylase in rat cerebellum with a new antiserum. Neuroscience 6(12): 2715-2735, 1981.

Oertel, W.H., Schmechel, D.E., Tappaz, M.L. and Kopin, I.J.: Production of a specific antiserum to rat brain glutamic acid decarboxylase by injection of an antigen-antibody complex. Neuroscience 6(12): 2689-2700, 1981.

Oertel, W.H., Schmechel, D.E., Brownstein, M.J., Tappaz, M.L., Ransom, D.H., and Kopin, I.J.: Decrease of glutamate decarboxylase (GAD)-immunoreactive nerve terminals in the substantia nigra after kainic acid lesion of the striatum. J. Histochem. Cytochem. 29(8): 977-980, 1981.

Oertel, W.H., Schmechel, D.E., Weise, V.K., Ransom, D.H., Tappaz, M.L., Krutzsch, H.C. and Kopin, I.J.: Comparison of cystein sulphinic acid decarboxylase isoenzymes and glutamic acid decarboxylase in rat liver and brain. Neuroscience 6(12): 2701-2714, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00405-03 LCS |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Aminergic Receptor Function | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Irwin J. Kopin OTHER: Joseph DiMicco Giora Feuerstein Zofia Zukowska-Grojec Mohamed Bayorh Robert Zerbe | Chief, LCS Staff Fellow Guest Worker Visiting Fellow Visiting Fellow Senior Staff Fellow | LCS NIMH LCS NIMH LCS NIMH LCS NIHM LCS NIHM LCS NIHM |
| COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Medicine | | |
| INSTITUTE AND LOCATION NIHM, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.0 | PROFESSIONAL: 1.5 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this project are to <u>assess changes</u> in properties (number, affinity) of <u>receptors</u> in response to alterations in the <u>environment</u> , drug administration, <u>stress</u> , or <u>hormones</u> , and to develop methods for assessing <u>receptor function</u> in intact animals, <u>in vivo</u> . This year the <u>sympatho-adrenal medullary</u> and <u>endocrine responses</u> to hemorrhage have been examined in detail and the essential role of <u>vasopressin</u> in the recovery from hypotension discovered. Studies on the role of <u>prostaglandins</u> in regulation of blood pressure have continued implicated in the pathogenesis of the hypertension in experimental animals. Studies of response in intact, awake animals to a variety of stressors and stimuli provide a necessary complement to studies in pithed rats (project 201 MH 401). In the intact animal various reflex mechanisms and hormonal responses not possible in pithed rats can be recruited to most challenges to the homeostatic mechanisms, and the role of the central nervous system in organizing and regulating these responses can be examined. | | |

PROJECT DESCRIPTION:

Hemorrhage elicits reflex sympatho-adrenal medullary release of catecholamines which are increased in plasma of bled anesthetized or awake rats. When angiotensin formation is blocked by the administration of captopril, recovery of anesthetized rats from a moderate hemorrhage is completely prevented; plasma epinephrine and vasopressin levels rise to higher levels, but appear to be ineffective in returning the blood pressure to normal levels (Drs. Zerbe, Feuerstein, and Kopin). Prostacyclin (PGI_2) infusion facilitates the recovery from hemorrhage in anesthetized intact or adrenalectomized-splanchnicectomized rats. When the adrenals are intact, PGI_2 enhances the catecholamine and vasopressin responses to hemorrhage; these appear sufficient since renin levels are not increased. When the adrenals are not intact, however, plasma epinephrine and vasopressin levels do not increase, but there is a marked increase in plasma renin (Drs. Feuerstein, Zerbe, Meyer, and Kopin). Other factors may also play a role in recovery from hemorrhage since in halothane-anesthetized, captopril-pretreated rats, naloxone facilitates recovery but does not enhance catecholamine responses.

In Brattleboro rats, which are unable to synthesize vasopressin, the recovery from hemorrhage is significantly blunted, although catecholamine and renin responses are enhanced. Normal rats have marked increases in vasopressin in response to hemorrhage, indicating that vasopressin is an important factor in the recovery process (Drs. Zerbe, Feuerstein, Meyer, and Kopin). Normal animals treated with vasopressin antagonist also have a delayed recovery from hemorrhage, supporting the important role of vasopressin (Drs. Kirtland, Zerbe, Bayorh, and Feuerstein).

The dysregulation produced by administration of phencyclidine corresponds with a rise in blood pressure and a fall in plasma vasopressin levels, possibly as an indirect effect of the pressor response (Drs. Zerbe, Bayorh, Quirion, and Kopin).

The roles of prostaglandins in regulating cardiovascular promotion are complex and poorly defined. Chronic treatment of SHR rats with arachidonic acid, the precursor of prostaglandins, retards the development of increased blood pressure and appears to diminish responsiveness to norepinephrine, as well as reduce sympathetic outflow from the central nervous system (Drs. Bayorh, Zukowska-Grojec, Feuerstein, and Kopin). Acute administration of arachidonic acid to conscious animals evokes greater hypotensive responses in SHR than WKY rats. Plasma catecholamines increase similarly in both strains, but 6-Keto PGF_1 levels are higher in SHR rats. Leukotriene D_4 produces hypotension in SHR, but not in WKY rats, which is partially blocked by pretreatment with indomethacin. These results suggest that the metabolism of prostaglandins may differ in SHR and WKY rats.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

Receptor function alteration is a potential factor in disease states and pharmacological responses. The mode of regulation of receptor function must be further understood to determine means of evaluating mechanisms of diseases on drug action.

PROPOSED COURSE:

Continued investigation of effects of stress, drugs, hormones, etc. on various aspects of receptor function (agonist binding secondary effects at a biochemical level e.g., cyclic AMP ion transport and net responses).

PUBLICATIONS:

Feuerstein, G., DiMicco, J.A., Ramu, A. and Kopin, I.J.: Effect of indomethacin on blood pressure and plasma catecholamine responses to acute endotoxemia. J. Phar. Pharmacol. 33: 576-579, 1981.

Feuerstein, G., Zerbe, R.L., Meyer, D.K., and Kopin, I.J.: Alteration of cardiovascular, neurogenic, and humoral responses to acute hypovolemic hypotension by administered prostacyclin. J. Cardiovasc. Pharmacol. 4 246-253, 1982.

Feuerstein, G., Zukowska-Grojec, Z., Bayorh, M.A., Krause, M., Utsonomiya, T., Lovenberg, W., and Kopin, I.J.: Effect of arachidonic acid on blood pressure, heart rate and plasma norepinephrine, epinephrine, 6-Keto - PGF₁ and TXB₂ conscious SHR and WKY rats. 4th International Symposium on SHR rats. In press.

Feuerstein, G., Zukowska-Grojec, Z., and Kopin, I.J.: Cardiovascular effects of leukotriene D₄ in SHR and WKY rats. Eur. J. Pharmacol. 76: 107-110, 1981.

Feuerstein, G., Zukowska-Grojec, Z., Krausz, M., Blank, M.L., Snyder, F., and Kopin, I.J.: Cardiovascular and sympathetic effects of 1-O-hexadecyl-2-acetyl-sn-glycero3-phosphocholine in conscious SHR and WKY rats. Clin. Exper. Herper-tension, 1982. In press.

Zerbe, R.L., Bayorh, M.A., and Feuerstein, G.: Vasopressin: An essential pressor factor for blood pressure recovery following hemorrhage. Peptides, 1982. In press.

Zerbe, R.L., Bayorh, M.A., and Kopin, I.J.: Cardiovascular, sympathetic and renin-angiotensin system responses to hemorrhage in vasopressin deficient rats. Endocrinology, 1982. In press.

Zerbe, R.L., Feuerstein, G., and Kopin, I.J.: Effect of captopril on cardiovascular, sympathetic and vasopressin responses to hemorrhage. Eur. J. Pharmacol. 72: 391-395, 1981.

Zerbe, R.L., and Susan, H.: A new met-enkephalin analogue suppresses plasma vasopressin in man. Peptides, 1982. In press.

Zukowska-Grojec, Z., Bayorh, M.A., Yaar, I., Feuerstein, G., and Kopin, I.J.: Leukotriene D₄: Divergent cardiovascular and sympathetic effects in SHR and WKY rats. Raven Press, 1982. In press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00351-08 LCS |
| PERIOD COVERED October 1, 1981 - September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Clinical Pharmacology of the Central Nervous System | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: OTHER: | M. H. Ebert R. S. Burns I. J. Kopin E. Gordon | Chief, Sec. on Exp. Therapeutics Staff Physician Chief, Lab. of Clinical Science Head, Unit on Clinical Biochemistry LCS NIMH LCS NIMH LCS NIMH LCS NIMH |
| COOPERATING UNITS (if any) Section on Medicine, LCS; Unit on Clinical Biochemistry, LCS | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Experimental Therapeutics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.0 | PROFESSIONAL: 1.5 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>The purpose of this study is to develop techniques for studying <u>central nervous system monoamine metabolism</u> in man, and to carry out <u>pharmacokinetic studies of psychoactive drugs</u>. Studies of the kinetics and clearance of <u>deuterated homovanillic acid (HVA)</u> from the circulation continued in Rhesus monkeys and patients. Problems with the application of this technique to study CNS dopamine turnover in man were resolved by further study of the kinetics and volume of distribution of the metabolite, HVA, before and after <u>probenecid</u> administration.</p> | | |

Project Description:

Objectives: (1) To develop techniques for studying kinetic parameters of central nervous system monoamine metabolism in man. (2) To carry out pharmacokinetic studies of psychoactive drugs. (3) To utilize these techniques to study patients with a variety of psychiatric, neuropsychiatric, and psychosomatic disorders.

Methods Employed: (1) Biochemical methods: fluorometric, gas chromatographic, and mass spectrometric methods are used for assay of endogenous catecholamine metabolites in tissues and body fluids. Mass spectrometric methods are used for assay of deuterium-labelled catecholamine metabolites administered exogenously or synthesized endogenously. Gas chromatographic and radio-immunoassay techniques are used for assay of drugs in body fluids. (2) Physiological methods: As the project progresses, an increasing number of metabolic experiments will be carried out in patients. However, as new procedures are being developed, appropriate experiments will be carried out in rat brain tissue and in primates using serial sampling of blood, cerebrospinal fluid, and urine as necessary. To facilitate these preclinical in vivo experiments on brain metabolism, a small primate metabolic laboratory has been developed with a capacity to maintain chronic collection of lateral ventricle or lumbar cerebrospinal fluid from chaired Rhesus monkeys for days or weeks at a time.

Major Findings: Unfortunately, the laboratory work in the section was compromised this year by the ADAMHA hiring freeze which eventually eliminated the support personnel in the section. The work on this project was diminished as a direct result, and studies of PCP kinetics and metabolism were delayed for 10 months.

Studies of the plasma and cerebrospinal fluid (CSF) kinetics of homovanillic acid (HVA) using deuterium-labelled HVA coupled with gas chromatography-mass spectrometry have continued in an effort to provide a better interpretation of HVA levels in lumbar CSF and plasma, and to develop a clinical method for measuring the dopamine production rate by the brain. Deuterated HVA with ^{14}C -inulin was injected into the lateral ventricle, the lumbar subarachnoid space (SAS), or a peripheral vein of the Rhesus monkey. The concentration of labelled HVA in plasma, urine, and lumbar CSF was measured to determine the mechanisms of elimination of HVA from ventricular and lumbar CSF as well as plasma, and the effect of probenecid on its transport at the choroid plexus. Deuterated HVA injected into the lateral ventricle rapidly appears in plasma (peak time 60-90 min.), suggesting that acute changes in brain dopamine turnover may be reflected in changes in the plasma HVA level. Ninety percent (90%) of HVA present in ventricular CSF is eliminated by a probenecid-sensitive mechanism at the choroid plexus with only an estimated 3% reaching the lumbar subarachnoid space. Levels of HVA in lumbar CSF are a function of both the delivery rate from the ventricle and the local clearance rate by diffusion across the blood-CSF barrier. The total body production rate of HVA is being compared in normal subjects and untreated patients with Parkinson's disease by analysis of the plasma kinetics of deuterated HVA, and by measurement of the urinary excretion of labelled and endogenous HVA. Assuming that peripheral dopamine systems are not affected in Parkinson's disease, a difference in the production rate of HVA will provide

a minimal estimate of the brain contribution total HVA production. The relative value of the 24 hour excretion rate of HVA and its level in plasma and lumbar CSF as an index of brain DA metabolism is also being assessed by comparing these patients with normal subjects. Lisuride, a prototypical dopaminergic ergot compound which is effective in the treatment of Parkinsonism, is now being used clinically in combination with the dopamine precursor L-DOPA. The presynaptic agonist effects of acute and subchronic oral lisuride on dopamine turnover is being studied in normal subjects by measuring the drug-induced changes in the concentration of HVA in plasma and the 24 hour excretion rate. The effect of chronic oral lisuride on the clearance rate and total production rate of HVA is being studied in Parkinsonian patients by determining the plasma kinetics of deuterated HVA on and off drug.

Significance of Biomedical Research: Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate of amine utilization. The development of more quantitative methods of determining central nervous system amine utilization in man is essential for testing hypotheses regarding the role of amines in psychiatric, medical, and neurological disorders, and assessing the effects of drugs on amines in the brain. Studies of the kinetics, metabolism, and behavioral effects of PCP bear directly on the psychiatric and neurological consequences of abuse of this drug.

Proposed Course: The metabolic techniques described above for the study of catecholamine metabolism are now being applied to studies in normal volunteers and patient groups.

Pharmacokinetic studies of PCP and its mono-hydroxy and di-hydroxy metabolites in normal volunteers will be concluded. The behavioral pharmacology of the mono-hydroxy and di-hydroxy metabolites will be studied in animals. Studies of the tissue distribution of PCP metabolites will be continued.

Publications:

Ebert, M.H., Van Vunakis, H., and Hawks, R.: Radioimmunoassays for Psychotropic Drugs. In Usdin, E. (Ed.): Clinical Pharmacology in Psychiatry North Holland, New York, Elsevier, 1981, pp. 73-94.

Lake, C.R., Gullner, H.G., Polinsky, R.J., Ebert, M.H., Ziegler M.G., and Bartter, F.C.: Essential hypertension: central and peripheral norepinephrine. Science. 211:955-957, 1981.

Elchisak, M.A., Polinsky, R.J., Ebert, M.H., and Kopin, I.J.: Kinetics of homovanillic acid and determination of its production rate in humans. J. Neurochem. 38(2):380-385, 1982.

Project No. Z01 MH 00351-08 LCS

Ebert, M.H., Kopin, I.J., Gordon, E.K., Oliver, J., Markey, S.P.: Stable Isotope Studies of Brain Catecholamine Metabolism. In Susan, A.B. (Ed.): Synthesis and Applications of Isotopically Labeled Compounds: Proceedings of the International Symposium 1982. Amsterdam, Elsevier, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00352-07 LCS |
| PERIOD COVERED October 1, 1981 - September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: OTHER: | M.H. Ebert W.H. Kaye P.R. Martin D. Goldman C.R. Merrill H. Weingartner I.J. Kopin L.E. Nee R.S. Burns S.P. Markey D. Pickar S. Higa A. Larsen | Chief, Sec. on Exp. Therapeutics Staff Psychiatrist Visiting Scientist Clinical Associate Sr. Research Scientist Research Psychologist Chief, Lab. of Clinical Science Clinical Social Worker Staff Physician Pharmacologist Staff Psychiatrist Visiting Fellow Visiting Fellow LCS NIMH LCS NIMH NIAAA LCS NIMH LGCB NIMH LPP NIMH LCS NIMH LCS NIMH LCS NIMH BPB NIMH LCS NIMH LET NINCDS |
| COOPERATING UNITS (if any) Section on Medicine, LCS; Laboratory of Psychology and Psychopathology, NIMH; Laboratory of General and Comparative Biochemistry, NIMH; NIAAA | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Section on Experimental Therapeutics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 2.5 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> Several neuropsychiatric syndromes including <u>Alzheimer's dementia</u> and <u>Korsakoff's psychosis</u> are being studied in terms of <u>memory function</u> and <u>possible pharmacological treatment</u>. The pathophysiology and neuropharmacology of <u>anorexia nervosa</u>, <u>Parkinson's disease</u>, and <u>essential tremor</u> are also under investigation. <u>Two-dimensional electrophoresis</u> has been developed into a quantitative tool for the identification of abnormal proteins or genetic markers in neuropsychiatric diseases. In a study of 300 lymphocyte proteins in Huntington's disease, no protein marker of the disease was identified, but six polymorphic proteins were discovered that can be utilized in linkage analysis. Patients with Korsakoff's psychosis exhibit specific deficits in episodic memory as opposed to semantic memory, and have decreased excretion of 5-hydroxy-melatonin, suggesting central adrenergic dysfunction. Patients with anorexia nervosa have persistent abnormalities in central nervous system norepinephrine metabolism, after weight restoration has occurred. </p> | | |

Project Description:

Objectives: The purpose of this study is to investigate several neuropsychiatric syndromes from the perspective of memory and intellectual function, neurotransmitter metabolism, neuroendocrine function, and possible pharmacological treatment. Currently under study are several dementias with known neuropathology, particularly Alzheimer's dementia and Korsakoff's dementia. Other diseases under intensive study are anorexia nervosa and Gilles de la Tourette's syndrome.

Methods Employed: Methods for studying information processing in neuropsychiatric disorders include learning tasks that permit the assessment of stimulus and modality specific information in processing (1) short-term memory; (2) long-term memory; (3) retrieval processing; (4) encoding; (5) the interrelationships of these processes; and (6) factors that might influence decay and interference of memory in these systems. Procedures are used to test free recall, serial learning, cued recall, and recognition memory.

One strategy for identifying neurotransmitter systems that may play a role in the pathogenesis of psychiatric or neurological symptoms is to study the clinical effects of a series of drugs having relatively specific stimulating or blocking effects on a particular neurotransmitter system. This approach has been profitably applied to research on affective disorders. We are pursuing a pilot investigation of this nature in patients with dementias of varying etiologies, focusing on the adrenergic and cholinergic system.

Methods used in neuroendocrine protocols are established techniques of studying plasma prolactin and growth hormone responses to the acute administration of drugs known to affect dopaminergic neurotransmission. From these endocrine responses, one can infer the functional state of dopaminergic transmission in the tuberoinfundibular systems. Methods used in Stage II drug trials are established methods for the evaluation of psychotropic drugs including double-blind design, appropriate rating scales, and placebo controls.

Methods used in studies of neurotransmitter metabolism are those methods outlined in project Z01 MH 00351-08 LCS.

Methods used to study protein abnormalities or search for genetic markers in psychiatric and neurological diseases involves the application of two-dimensional polyacrylamide gel electrophoresis of proteins. These two-dimensional gels can be studied and quantitatively analyzed either by silver staining of the proteins present or by labeling the proteins in biological sample and performing radioautograms. Image processing computer systems are used to identify and quantitate the individual proteins appearing on the gel.

Major Findings: A collaborative research effort with Dr. Carl Merrill of LGCB, NIMH has continued this year, and the laboratory supporting this research has continued to receive support from the NIAAA. In these studies, two-dimensional electrophoresis (2DE) has been developed into a quantitative tool for the identification of molecular markers and probes for neuropsychiatric diseases. To perform this type of analysis, more than 1000 cellular proteins or a somewhat smaller number of proteins in a biological fluid are resolved on the two-dimensional electrophoretogram, visualized by staining or

autoradiography, and then measured for variation in spot density or position with the aid of a computer.

A major effort was made in the past year to further improve this technology in several areas. The ultrasensitive silver stain for proteins developed by Dr. Merrill has been simplified and shown to possess sufficient linearity and reproducibility for quantitative use. The silver stain has also been shown to be effective for detecting trace amounts of DNA, RNA, and lipopolysaccharides.

In collaboration with Wayne Rasband of RSB, NIMH, the computer analysis of electrophoretic patterns has been refined using an RSB PDP 11/60 computer so that measurements are made more accurately and quickly. A larger computer, a VAX-750, is now being programmed for gel analysis with the assistance of Dr. Mark Miller of NCI.

Lesch-Nyhan syndrome is an X-linked recessive disease caused by a deficiency of hypoxanthine-guanine phosphoribosyltransferase. It results in spasticity, hyperuricemia, and self-mutilation. Quantitative differences in the activities of several peripheral cellular enzymes have been shown to be associated with it. One category of protein variation which might be detected with 2DE is a pattern of protein modulations secondary to a primary metabolic disturbance. In a study of Lesch-Nyhan patients, 400 lymphocyte proteins from each individual were measured and 11 proteins were identified that were consistently modulated with the disease.

Another category of polypeptide variation which may be observed with 2DE is the association of normal polymorphic protein variants with a disease locus within a pedigree (because of genetic linkage and a low rate of recombination) or within the population (because of linkage disequilibrium). A study on Huntington's disease (HD) was carried out this year to search for genetic markers of the disease. The study was done on 28 individuals, including 13 with HD, 2 at risk for HD, and neurological controls. A quantitative analysis of the 306 most dense lymphocyte proteins failed to show any correlation with HD. This result supports recent studies which have failed to confirm earlier reports of membrane and other abnormalities in the peripheral cells of individuals with HD. In the course of this study, a number of polymorphisms were discovered. For six polymorphic proteins, three phenotypes were observed in the population we studied. Our mathematical analysis indicates that the number of polymorphisms available by 2DE will make significant impact on linkage analysis. (Drs. Goldman, Merrill, Ebert.)

Neurochemical and neuropharmacological studies of Alzheimer's dementia are concluding this year. A chronic treatment study of lecithin and an anticholinesterase (tetrahydroaminoacridine), administered in combination to increase acetylcholine turnover in the central nervous system, has not proved to be efficacious in improving memory function in Alzheimer patients. (Drs. Kaye, Ebert, Weingartner, Gillin.)

A genetic study of a Canadian family containing 45 members affected with Alzheimer's disease was completed this year. Ancestors were traced through 8 generations and 51 members were examined at the National Institute of Mental

Health. Pedigree analysis indicated autosomal dominant inheritance. This family will be further studied with genetic marker techniques. (Ms. Nee, Drs. Polinsky, Ebert.)

A study of the relationship between neurochemical changes within the central nervous system and memory function in Korsakoff's psychosis is underway. Although Korsakoff patients perform as poorly on recall tasks (episodic memory) as patients with early Alzheimer's disease, studies in collaboration with Dr. Weingartner (LPP, NIMH) demonstrate that different mechanisms underlie their memory failure. For example, Korsakoff patients are able to utilize cues such as the organization of material to be remembered in order to aid recall, since their ability to access knowledge structures in memory (semantic memory) is relatively intact compared to individuals with early Alzheimer's disease. Clinical assessment of patients with Korsakoff's psychosis indicate the presence of residual neurological signs several years after thiamine treatment of the acute (Wernicke) stage of the illness. Measurement of 6-hydroxy-melatonin in the urine of these patients (in collaboration with Drs. Higa and Markey, LCS) demonstrate substantial reduction in excretion of this major metabolite of melatonin. This biochemical finding suggests central adrenergic dysfunction in Korsakoff's psychosis. Analysis of CSF and plasma catecholamine metabolites are in progress, and neuropharmacological assessment of autonomic dysfunction is planned. An animal model of thiamine deficiency which neuropathologically resembles Korsakoff's dementia is being adapted to further investigate neurochemical abnormalities. (Drs. Martin, Ebert.)

Clinical studies of anorexia nervosa have continued to focus on defining psychological and neurochemical changes that take place during the evolution of the chronic illness. A clinical design is utilized in which we study underweight anorexics, the same women after weight restoration, a separate group of women who were once underweight with anorexia but have been weight recovered for at least 20 months, and normal control women. Underweight anorexics have low CSF 5HIAA and HVA, indicating decreased brain serotonin and dopamine turnover that corrects with weight recovery. Likewise underweight anorexics have levels of CSF total opioids that are several times higher than normal levels. In contrast, long term weight recovered anorexics continue to have disturbances in central and peripheral norepinephrine (NE) metabolism compared to normals (decreased plasma and CSF NE and NE metabolites). This abnormality may bear some relationship to chronic changes in appetite and metabolic regulation. (Drs. Kaye, Ebert, Pickar.)

A clinical study of untreated Parkinsonian patients who have associated hypertension, hypotension, or a positive family history for Parkinsonism is being carried out. (Drs. Burns, Ebert, Kopin.) Although the motor signs are uniform in the syndrome of Parkinsonism, involvement of the autonomic nervous system and other functional brain systems may vary greatly. We are currently trying to characterize biochemically the adrenergic system as well as the dopamine system in untreated Parkinsonian patients with associated findings in an effort to differentiate the subtypes of this syndrome. This may be of diagnostic as well as predictive value with respect to clinical response and side effects of drug therapy. Catecholamines and catecholamine metabolites as well as 6-OH-melatonin are being assessed.

We are currently investigating biochemically the adrenergic system in untreated, essential tremor patients and studying the effect of clonidine on tremor in this disorder. (Drs. Burns, Larsen, Martin, Ebert, Kopin.) The concentration of catecholamines and their metabolites in body fluids including CSF is being used to assess adrenergic system function and drug effect. Computer analyses of accelerometer recordings are being used to measure the drug's effect on tremor amplitude. Based upon a pilot study involving a small group of patients, clonidine appears to be therapeutically effective in decreasing the tremor amplitude in relatively low doses without side effects.

Significance of Biomedical Research: In recent years interest has increased in the biochemical aspects of learning and memory. Neuropharmacological aspects of memory function have also become a focus of interest. Most of the published studies in this area are in animals. We hope to extend knowledge about pharmacological and biochemical aspects of memory function by studying several diseases in which disordered memory is a primary symptom, and in which there is existing knowledge about anatomical and biochemical pathology. Gilles de la Tourette's syndrome is a neuropsychiatric syndrome whose pathophysiology is poorly understood. The symptomatology appears to be responsive to drugs which block dopamine receptors. By conducting new drug trials and generating data on cognitive and linguistic function, monoamine metabolism, and neurological endocrine function in this illness, we hope to expand knowledge about its pathophysiology. Anorexia nervosa is another neuropsychiatric syndrome in which there is little published information concerning pharmacological treatment and neurotransmitter metabolism. It is clearly established in the animal literature that monoamine systems in the hypothalamus play an important role in the normal regulation of appetite.

Proposed Course: Clinical applications of two-dimensional electrophoresis will include further family studies to describe additional polymorphisms that can be used for linkage analysis. Two-dimensional electrophoresis of cerebrospinal fluid will be applied to the study of several genetic or degenerative neurological diseases. No further clinical trials of cholinergic agents in Alzheimer's disease are planned. Further studies of central noradrenergic function in Korsakoff's psychosis are planned. Neurochemical studies of opiate, noradrenergic, and central vasopressin systems in anorexia nervosa will continue with the goal of determining whether these systems play a role in the chronic course of the illness. Studies of bulimia, a parallel disorder of eating behavior, have begun. Pilot neurochemical studies of Parkinson's disease and essential tremor described above will be concluded.

Publications:

Martin, P.R.: The human genetics of alcoholism. Substance and Alcohol Actions/Misuse 2. 389-406, 1981.

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Kaye, W.H., Sitaram, N., Weingartner, H., Ebert, M.H., Smallberg, S., Gillin, J.C.: Modest facilitation of memory in dementia with combined lecithin and anticholinesterase treatment. Biol Psychiatry. 17(2)275-280, 1982.

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Small, A.C., Teagne, L., Madero, J., Ebert, M.H.: A comparison of anorectics and schizophrenics on psychodiagnostic measures. J. Eating Disorders. In Press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00424-07 LCS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981, through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Biological Active Peptides in the Brain | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI's:</td> <td style="width: 40%;">M. Brownstein</td> <td style="width: 40%;">N. Zamir</td> <td style="width: 10%;">LCS NIMH</td> </tr> <tr> <td></td> <td>L. Eiden</td> <td>T. Hokfelt</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>R. Pruss</td> <td>G. Norell</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>N. Sherwood</td> <td>M. Palkovits</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>F. Douglas</td> <td>E. Mezey</td> <td>LCS NIMH</td> </tr> <tr> <td>Other:</td> <td colspan="2">E. Schiffman, G. Vasanthakumar, D. Pencev</td> <td>LDBA NIDR</td> </tr> <tr> <td></td> <td colspan="2">D. Klein, A. Namboudiri</td> <td>LDN CH</td> </tr> <tr> <td></td> <td colspan="2">M. Whitnall, H. Gainer, J. Russell</td> <td>LDN CH</td> </tr> <tr> <td></td> <td colspan="2">R. Eskay</td> <td>NINCDS</td> </tr> <tr> <td></td> <td colspan="2">H. Schmale, R. Ivell, D. Richter</td> <td>U. Hamburg</td> </tr> <tr> <td></td> <td colspan="2">E. Herbert, M. Comb, P. Seeburg</td> <td>U. Oregon</td> </tr> <tr> <td></td> <td colspan="2">T. Williams, I. Kiss</td> <td>U. Iowa</td> </tr> <tr> <td></td> <td colspan="3">see continuation page</td> </tr> </table> | | | PI's: | M. Brownstein | N. Zamir | LCS NIMH | | L. Eiden | T. Hokfelt | LCS NIMH | | R. Pruss | G. Norell | LCS NIMH | | N. Sherwood | M. Palkovits | LCS NIMH | | F. Douglas | E. Mezey | LCS NIMH | Other: | E. Schiffman, G. Vasanthakumar, D. Pencev | | LDBA NIDR | | D. Klein, A. Namboudiri | | LDN CH | | M. Whitnall, H. Gainer, J. Russell | | LDN CH | | R. Eskay | | NINCDS | | H. Schmale, R. Ivell, D. Richter | | U. Hamburg | | E. Herbert, M. Comb, P. Seeburg | | U. Oregon | | T. Williams, I. Kiss | | U. Iowa | | see continuation page | | |
| PI's: | M. Brownstein | N. Zamir | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | L. Eiden | T. Hokfelt | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | R. Pruss | G. Norell | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | N. Sherwood | M. Palkovits | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | F. Douglas | E. Mezey | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Other: | E. Schiffman, G. Vasanthakumar, D. Pencev | | LDBA NIDR | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | D. Klein, A. Namboudiri | | LDN CH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | M. Whitnall, H. Gainer, J. Russell | | LDN CH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | R. Eskay | | NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | H. Schmale, R. Ivell, D. Richter | | U. Hamburg | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | E. Herbert, M. Comb, P. Seeburg | | U. Oregon | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | T. Williams, I. Kiss | | U. Iowa | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | see continuation page | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) LDBA NIDR; LDN CH; NINCDS; U. Hamburg; U. Oregon; U. Iowa; St. Louis U.; Columbia U.; Mt. Sinai; USUHS; U. California, Riverside; Johns Hopkins; U. Virginia; U.C. London; Harvard; U. Colorado; Salk Inst.; BPB NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Office of the Chief | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 5.75 | PROFESSIONAL: 5.75 | OTHER: 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) We have continued to study the distribution of <u>peptide-containing cells</u> in the central nervous system, the biosynthesis of biologically active peptides, and the factors that regulate <u>peptide secretion</u> . Our studies of a number of peptides have contributed to a better understanding of the <u>cell biology</u> of peptidergic neurons and of their role in the brain. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project No. Z01 MH 00424-07 LCS

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project (continued)

| | |
|-------------------------------|--------------------------|
| L. Zaborszky | U. Virginia |
| M. Beinfeld | St. Louis University |
| E. Zimmerman, G. Nalaver | Columbia University |
| D. Kreiger | Mt. Sinai |
| H. Faden | USUHS |
| S. Spindler | U. California, Riverside |
| H. Sun | Johns Hopkins |
| M. Thorner | U. Virginia |
| M. Raff, R. Mirsky | U.C. London |
| R. Linck | Harvard |
| J. Ruth | U. Colorado |
| W. Vale, J. Rivier, J. Speiss | Salk Institute |
| L. Skirboll | BPB NIMH |

PROJECT DESCRIPTION:

Drs. Brownstein, Vasanthakumar, Pencev, and Schiffman have continued to work on the purification of the antichemotactic substance(s) elaborated by mouse fibrosarcomas. The development of a bioassay based on the use of human neutrophils has expedited this project. Three peaks of activity have been resolved by HPLC. Two of these are fairly clean.

Drs. Brownstein, Namboudiri, and Klein have succeeded in partially purifying the pineal serotonin N-acetyltransferase by a combination of affinity and high performance liquid chromatographic procedures.

Drs. Brownstein, Schmale, Ivell, and Richter have isolated mRNA from samples of rat supraoptic nucleus and have translated vasopressin mRNA.

Drs. Sherwood, Eiden, Brownstein, Speiss, Rivier, and Vale have isolated and characterized the salmon spawning factor. This is a decapeptide with 8 of 10 amino acids identical to those of mammalian GnRH.

Drs. Eiden and Ruth have characterized the effects of enkephalins on the isolated atrium.

Drs. Eiden, Comb, and Herbert have isolated mRNA from human pheochromocytoma and have prepared a cDNA library. Using an oligonucleotide probe they have isolated cDNA to enkephalin mRNA and have succeeded in sequencing the 50K dalton preproenkephalin molecule.

Drs. Palkovits, Douglas, Eiden, and Beinfeld have continued to study the distribution of biologically active peptides in the CNS and to characterize peptidergic pathways there. Drs. Palkovits, Kiss, Williams, Zaborsky, and Mezey have studied peptidergic nerve endings in the median eminence and nucleus tractus solitarius by means of EM immunocytochemistry.

Drs. Hökfelt and Skirboll have studied descending inputs to the spinal cord by retrograde injection of fluorescent dyes and immunocytochemistry. Drs. Hökfelt, Faden, and Brownstein have investigated the effects of naloxone and TRH on symptoms of spinal cord trauma.

Dr. Pruss has succeeded in preparing monoclonal antibodies against chromaffin granule proteins. She is currently characterizing their specificities and will use them in the long run to study granule assembly and peptide packaging.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Nerve cells use chemical "transmitters" to communicate with one another and with other target cells. Changes in transmitter biosynthesis, release, and/or metabolism have been suggested to result in nervous and mental disorders. Death of dopaminergic neurons in the substantia nigra, for example, is associated with the symptoms of Parkinson's disease. In the last ten years the number of putative neurotransmitters has increased by a factor of four or five. Most of the newly detected chemical messengers are peptides. Our knowledge of the anatomy, physiology, and pharmacology of peptidergic neurons is comparatively incomplete at present; indeed, it is clear that many biologically active peptides remain to be isolated and characterized. The work outlined above is principally devoted to improving our understanding of peptide secreting nerve cells. To the extent that we

understand these cells, we can formulate better hypotheses about their role in causing disease.

PROPOSED COURSE:

The work outlined above is still in progress and will be continued.

PUBLICATIONS:

Eskay, R.L., Furness, J.B., and Long, R.T.: Substance P activity in the bullfrog retina: localization and identification in several vertebrate species. *Science* 212: 1049-1051, 1981.

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Meyer, D.K., Beinfeld, M.C., Oertel, W.H., and Brownstein, M.J.: Origin of the cholecystokinin-containing fibers in the rat caudatoputamen. *Science* 215: 187-188, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00271 13 LCS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Disposition and Metabolism of 3-Methoxy-4-Hydroxyphenyl Glycol (MHPG) in Humans | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 60%;">Irwin J. Kopin</td> <td style="width: 30%;">Chief, LCS</td> <td style="width: 10%;">LCS NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Edna K. Gordon</td> <td>Chemist</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Michael H. Ebert</td> <td>Chief, Section on Experimental Therapeutics</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Jerry A. Oliver</td> <td>Chemist</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Robert L. Sherman</td> <td>Chemist</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Sanford P. Markey</td> <td>Pharmacologist</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>David Jimerson</td> <td>Staff Physician</td> <td>LCS NIMH</td> </tr> </table> | | | PI: | Irwin J. Kopin | Chief, LCS | LCS NIMH | OTHER: | Edna K. Gordon | Chemist | LCS NIMH | | Michael H. Ebert | Chief, Section on Experimental Therapeutics | LCS NIMH | | Jerry A. Oliver | Chemist | LCS NIMH | | Robert L. Sherman | Chemist | LCS NIMH | | Sanford P. Markey | Pharmacologist | LCS NIMH | | David Jimerson | Staff Physician | LCS NIMH |
| PI: | Irwin J. Kopin | Chief, LCS | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| OTHER: | Edna K. Gordon | Chemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Michael H. Ebert | Chief, Section on Experimental Therapeutics | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Jerry A. Oliver | Chemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Robert L. Sherman | Chemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Sanford P. Markey | Pharmacologist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | David Jimerson | Staff Physician | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Office of the Chief | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Gas liquid chromatography-mass spectrometry (GLC-MS)</u> and <u>high pressure liquid chromatography (HPLC)</u> are used to isolate and measure <u>homovanillic acid (HVA)</u> , <u>3-methoxy, 4-hydroxyphenylglycol (MHPG)</u> and <u>vanillyl mandelic acid (VMA)</u> and other catecholamine metabolites in the urine of controls and patients with <u>orthostatic hypotension</u> as well as with various <u>neurologic</u> and <u>mental</u> disorders. Deuterated D(-) MHPG has been injected intravenously into human controls quickly and as a slow infusion. Blood and plasma values for endogenous and deuterated MHPG and VMA have been analyzed to study the kinetics of conversion of MHPG to VMA and the rates of elimination of the compounds and CSF examined to determine penetration into this fluid compartment. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

PROJECT DESCRIPTION:

Deuterated MHPG injected as a bolus or administered as a slow infusion over 3-4 hours is excreted in urine as a conjugate of MHPG or converted to VMA. Kinetic analysis of plasma free MHPG indicates that unconjugated plasma MHPG is a major transitional metabolite which accounts for about 2/3 of the total urinary catecholamine metabolites. About half of the MHPG in plasma is excreted as a conjugate whereas the remainder is converted to VMA and accounts for about half of the VMA excreted in the urine. Both conjugated MHPG and VMA are derived from sources independent of plasma MHPG e.g., DHPG. (Drs. Ebert, Markey, Blombery, Mrs. Gordon, Mr. Oliver and Dr. Kopin).

Although there is a popular concept that total urinary MHPG may reflect brain catecholamine metabolism, we have obtained good evidence which indicates that less than 30% of urinary MHPG is derived from brain.

Plasma MHPG levels may however provide a useful index of cumulative sympathetic activity in the whole body. The levels of MHPG in plasma are significantly greater in depressed patients who fail to suppress plasma cortisol levels in response to dexamethasone than in patients who show normal suppression (Drs. Jimerson, Insel, Reus, and Kopin). There is a high concordance between MHPG plasma levels of normal monozygotic twins, although levels of the metabolite vary in patients with rapidly cycling manic-depressive disorder - being higher during mania than during depression (Drs. Jimerson, Nurnberger, Post, Gershon, and Kopin).

CSF obtained at varying intervals after initiation of a constant infusion of deuterated MHPG contains the labelled compound at concentrations which approach those in plasma by 4 hours, indicating that there is an exchange of plasma and CSF MHPG (Drs. Ebert, Kopin, Mrs. Gordon and Mr. Oliver). This is consistent with the observation that plasma and CSF levels of free MHPG are highly correlated. CSF levels are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from the tumor of the adrenal medulla. This is explained by a plasma and CSF two-compartment system with similar rate constants for entry into and exit from the CSF compartment. MHPG formed, but not metabolized, in the central nervous system maintains CSF levels of MHPG at a constant increment over those in plasma. Estimation of this increment provides the best available index of formation of MHPG in the central nervous system (Drs. Kopin, Polinsky, Mrs. Gordon and Dr. Jimerson).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

MHPG is a major catecholamine metabolite, particularly in brain and CSF. Its rate of excretion has been claimed (incorrectly) to reflect brain adrenergic activity. These studies are aimed at defining the usefulness of MHPG levels and excretion as an index of adrenergic activity.

PROPOSED COURSE:

Continued studies of MHPG plasma CSF levels and urinary excretion in disease states and during drug administration.

PUBLICATIONS:

Kopin, I.J.: Assessing norepinephrine metabolism in human brain: Past, present, and future. In Matthysse, S. (Ed.): Psychiatry and the Biology of the Human Brain. New York, Elsevier North Holland, 1981, pp. 89-101.

Jimerson, D.C., Nurnberger, J.I., Post, R.M., Gershon, E.S. and Kopin, I.J.: Plasma MHPG in rapid cyclers and healthy twins. Arch. Gen. Psychiatry 38: 1287-1290, 1981.

Jimerson, D.C., Markey, S.P., Oliver, J.A. and Kopin, I.J.: Simultaneous measurement of plasma 4-hydroxy-3-methoxyphenylethylene glycol and 3,4-dihydroxyphenylethylene glycol by gas chromatography-mass spectrometry. Biomed. Mass Spectrom. 8(6): 256-259, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00274-08 LCS |
| PERIOD COVERED October 1, 1981, through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Methods of Ionization in Mass Spectrometry | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Sanford P. Markey Pharmacologist LCS NIMH Other: Leonid Kelner Visiting Scientist BEIB DRS Fred P. Abramson Visiting Scientist CPB NCI | | |
| COOPERATING UNITS (if any) Biomedical Engineering and Instrumentation Branch, DRS: Clinical Pharmacology Branch, NCI | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Office of the Chief | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.5 | PROFESSIONAL: 1.0 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Alternate ion sources for enhanced sensitivity and performance of analytical mass spectrometers have been constructed and tested. A means for detecting specific nuclides (¹⁴ C, ¹³ C, ¹⁴ N, ¹⁵ N, etc.) as compounds elute from a capillary gas chromatograph has been found using a microwave discharge interface. | | |

PROJECT DESCRIPTION:

The collaborative project to develop a mass spectrometer suitable for testing various ion sources has been completed (see Project No. Z01 RS 10073-03 BEI) and will be applied to determine the value of various ionization means for analytical mass spectrometry.

Efforts to use discharge ionization to destructively react and ionize organic compounds failed due to the inability of the quadrupole mass analyzer to resolve high energy ion beams. This effort will be transferred to the newly built instrument above which can select ions of appropriate energy for mass analysis.

An alternate approach to the same question has led to the successful development of a microwave discharge interface between a capillary gc and a conventional mass spectrometer. Gc effluent is mixed with oxygen or other reactant gas, passed through the discharge tube at reduced pressure, and admitted into the ion source of a conventional mass spectrometer. All organic molecules are converted to di- to triatomic products (CO , CO_2 , N_2 , NO , SO_2 , etc.) which can readily be detected and quantified. This system is currently being refined for routine use in metabolic studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Structure elucidation of unknown and specific detection of known compounds remain difficult areas of biomedical research. The mass spectrometric methods being pursued in this project are intended to focus the specificity of mass spectrometry toward the solution of currently insolvable problems.

PROPOSED COURSE:

Evaluation of various ion sources suitable for liquid chromatography-mass spectrometry and/or the analysis of high molecular weight (1000-10,000 daltons) polar compounds will be assessed with a newly constructed experimental mass spectrometer system. The microwave discharge interface will be refined for routine laboratory use.

PUBLICATIONS:

Markey, S.P.: Quantitative mass spectrometry. Biomed. Mass Spectrom. 8: 426-430, 1981.

Abramson, F.P. and Markey, S.P.: A chemical reaction capillary gc/ms interface. Proc. 30th Ann. Conf. Mass Spectrometry and Allied Topics, in press, 1982.

Markey, S.P. and Abramson, F.P.: Element and isotope specific detection by capillary gas chromatography-mass spectrometry using a microwave discharge interface. Proc. International Symposium on Synthesis and Applications of Isotopically Labeled Compounds, in press, 1982.

Markey, S.P. and Abramson, F.P.: Capillary gas chromatography-mass spectrometry with a microwave discharge interface for radioactive carbon-containing compounds. Anal. Chem., in press, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00275-04 LCS |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Release and Turnover of Catecholamine Metabolites in Human Subjects | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Irwin J. Kopin Chief, LCS LCS NIMH | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Clinical Science | | |
| SECTION Office of the Chief | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| CHICK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been terminated as a separate entity and is now combined with project Z01 MH 00403-09 because of the similarity and overlap in objectives, techniques and personnel. | | |

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|--|--|---|----------|-------------------|----------------|----------|--------|----------------|-----------------|----------|--|-----------------|------------------------|----------|--|------------------|-------------------|----------|--|-----------------|--------------------|----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00276-03 LCS | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981, through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Metabolism of Melatonin | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 40%;">Sanford P. Markey</td> <td style="width: 30%;">Pharmacologist</td> <td style="width: 10%;">LCS NIMH</td> </tr> <tr> <td>Other:</td> <td>Sadayoshi Higa</td> <td>Visiting Fellow</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Merrily A. Poth</td> <td>Asst. Prof. Pediatrics</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Phyllis E. Buell</td> <td>Student Scientist</td> <td>LCS NIMH</td> </tr> <tr> <td></td> <td>Peter R. Martin</td> <td>Clinical Associate</td> <td>LCS NIMH</td> </tr> </table> | | | PI: | Sanford P. Markey | Pharmacologist | LCS NIMH | Other: | Sadayoshi Higa | Visiting Fellow | LCS NIMH | | Merrily A. Poth | Asst. Prof. Pediatrics | LCS NIMH | | Phyllis E. Buell | Student Scientist | LCS NIMH | | Peter R. Martin | Clinical Associate | LCS NIMH |
| PI: | Sanford P. Markey | Pharmacologist | LCS NIMH | | | | | | | | | | | | | | | | | | | |
| Other: | Sadayoshi Higa | Visiting Fellow | LCS NIMH | | | | | | | | | | | | | | | | | | | |
| | Merrily A. Poth | Asst. Prof. Pediatrics | LCS NIMH | | | | | | | | | | | | | | | | | | | |
| | Phyllis E. Buell | Student Scientist | LCS NIMH | | | | | | | | | | | | | | | | | | | |
| | Peter R. Martin | Clinical Associate | LCS NIMH | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Sections on Medicine and Experimental Therapeutics, LCS; Department of Pediatrics, USUHS | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Office of the Chief | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYLARS: 2.0 | PROFESSIONAL: 1.5 | OTHER: 0.5 | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The major urinary metabolite of the pineal hormone melatonin, <u>6-hydroxymelatonin</u> is being quantified by gas chromatography-negative chemical ionization mass spectrometry. Urinary excretion rates of this metabolite are being used to determine the possible role of the pineal gland in the development of puberty in normal children or during normal menstrual cycles. Longitudinal studies on prepubertal girls are in progress. A study on seasonal variations in pineal function is in progress. The use of pineal hormone metabolites as an index of noradrenergic function in various clinical states is being pursued. | | | | | | | | | | | | | | | | | | | | | | |

PROJECT DESCRIPTION:

The function of the pineal gland in normal human physiology remains unknown. Measurement of the principal pineal hormone product melatonin, or its major metabolite, conjugated 6-hydroxymelatonin are being used to test various hypotheses of the role of the pineal gland. We have demonstrated that plasma levels of melatonin are most useful to reflect short-term regulation or pharmacological manipulation of the pineal gland, and that urinary metabolite levels are preferred for questions of daily pineal gland production.

We have used the urinary metabolite assay to demonstrate that extrapineal production of melatonin in rats is less than 1-3% that of the pineal gland. Pinealectomy and the measurement of urinary melatonin metabolite production demonstrated that negligible quantities of melatonin entering the general circulation could be derived from extrapineal sites, a finding which confirmed our studies in monkeys, but which stands in marked contrast to the claims of other scientists. It had become generally accepted that the rat produced melatonin in the retina and harderian gland, and this accounted for the melatonin detectable in plasma by radioimmunoassay following pinealectomy. We suggest that pinealectomized rat plasma should be a suitable procedural blank for the radioimmunoassays of melatonin.

Previously, we studied urinary excretion rates of melatonin metabolites for children at various stages of pubertal development and found a significant increase coincident with the onset of breast development in girls. A longitudinal study of young girls is in progress and will require another 1-2 years to complete.

A study of the rate of excretion of melatonin metabolites during the menstrual cycle is also in progress. Plasma hormones and basal body temperatures are being determined to correlate any changes in pineal function with other hormonal changes. As a control for these studies, urinary excretion rates are being determined for men.

Melatonin is known to effect reproductive stages in seasonally breeding animals. Thus, the question of change in melatonin production throughout the year is being determined in a group of men.

Studies on patients with decreased β -adrenergic activity are being continued. Patients with Korsakoff's syndrome exhibit diminished metabolite excretion relative to controls. The effect of clonidine is to further diminish melatonin formation in a dose related fashion. An animal model for Korsakoff's syndrome is being tested.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Studies on the normal physiologic role of melatonin in human biochemistry are lacking. This project is intended to gather baseline data and define the possible role of melatonin in several of its most frequently cited functions. Postulates regarding altered melatonin production as a consequence of altered circadian function and their relation to mental health require these data.

PROPOSED COURSE:

See last four paragraphs.

PUBLICATIONS:

Tetsuo, M., Polinsky, R.J., Markey, S.P., and Kopin, I.J.: Urinary 6-hydroxymelatonin excretion in patients with orthostatic hypotension. J. Clin. Endocrinol. Metab. 53: 607-610, 1981.

Markey, S.P., Colburn, R.W., and Johannessen, J.N.: Efficient extraction and mass spectrometric assay of serotonin in biological fluids. Biomed Mass Spectrom. 8: 301-304, 1981.

Tetsuo, M., Perlow, M.J., Mishkin, M., and Markey, S.P.: Light exposure reduces and pinealectomy virtually stops urinary excretion of 6-hydroxymelatonin by rhesus monkeys. Endocrinology 110, 997-1003, 1982.

Tetsuo, M., Poth, M., and Markey, S.P.: Melatonin metabolite excretion during childhood and puberty. J. Clin. Endocrinol. Metab. in press, 1982.

Markey, S.P. and Buell, P.E.: Pinealectomy abolishes 6-hydroxymelatonin excretion by male rats. Endocrinology, in press, 1982.

Taylor, P.L. and Markey, S.P.: High resolution gas chromatography-negative chemical ionization mass spectrometry of indole amines. Proc. 29th Ann. Conf. Mass Spectrometry and Allied Topics, 55-58, 1981.

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|---|--|---|-----------------------|----------------|----------|----------------------------|-----------------|----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00277-03 LCS | | | | | | |
| PERIOD COVERED October 1, 1981, through September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Synthesis of Stable Isotope Labelled Compounds | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Sanford P. Markey</td> <td style="width: 33%;">Pharmacologist</td> <td style="width: 33%;">LCS NIMH</td> </tr> <tr> <td>Other: Wilfried J.W. Mayer</td> <td>Visiting Fellow</td> <td>LCS NIMH</td> </tr> </table> | | | PI: Sanford P. Markey | Pharmacologist | LCS NIMH | Other: Wilfried J.W. Mayer | Visiting Fellow | LCS NIMH |
| PI: Sanford P. Markey | Pharmacologist | LCS NIMH | | | | | | |
| Other: Wilfried J.W. Mayer | Visiting Fellow | LCS NIMH | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Science | | | | | | | | |
| SECTION Office of the Chief | | | | | | | | |
| INSTITUTE AND LOCATION NIH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 1.1 | OTHER: 0.1 | | | | | | |
| CHLCK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Syntheses of <u>oxygen-18</u> and <u>deuterium</u> labelled compounds are being developed for clinical studies on their metabolic fate and for internal standards in mass spectral assays. Synthesis of <u>dioxygen-18</u> labelled 3,4 dihydroxyphenylethylene-glycol (DHPG) has been completed in quantities sufficient for animal and human studies. Analogs of melatonin and its metabolites are being synthesized. | | | | | | | | |

PROJECT DESCRIPTION:

Stable isotope analogs or isotopimers of biogenic amines are being used for human clinical research (Z01 MH 00275-05 LCS) to determine the metabolism and kinetics of distribution and excretion of neurotransmitters. Common to many of the compounds of interest are catechol oxygens, generally enzymatically monomethylated during metabolism. Thus, routes of synthesis of oxygen-18 labelled catechols or monodeuteromethylated catechols are being pursued.

Using the previously described route of test scale synthesis, 150 mg of the norepinephrine metabolite 3,4-dihydroxy- $^{18}\text{O}_2$ -phenylethyleneglycol has been prepared for animal and human pharmacokinetic studies.

Small scale syntheses of the ethyl homolog of melatonin (5-ethoxy-N-acetyl-tryptamine), and 5-ethoxyindoleacetic acid have been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Stable isotope labeled compounds are required as internal standards for mass spectrometric analyses, and more importantly as in vivo tracers of the metabolic fate of normal metabolic intermediates. Many hypotheses of organic mental disfunction postulate altered production, release, or metabolism of neurotransmitters. This project is intended to provide the required chemical materials to test these hypotheses in human subjects without the risks attendant radiolabeled compounds.

PROPOSED COURSE:

Small pilot scale syntheses of deuterium labeled melatonin metabolites (kynureninamines) will be undertaken. An evaluation of $^{18}\text{O}_2$ -DHPG metabolism in rats will be performed prior to using the synthesized material in humans.

PUBLICATIONS:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH-00247-07 |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Patterns of Psychological Functioning in Children with Endocrine Abnormalities | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Jerome H. Blue | Staff Fellow LDP NIMH |
| OTHER: | Thomas M. Achenbach, Ph.D. | Research Psychologist Univ. of Vermont Burlington, Vt. |
| | Craig Edelbrock, Ph.D. | Research Psychologist Boys Town, Ne. |
| | Roger Johnsonbaugh, M.D. | Pediatric Endocrinologist National Naval Medical Center |
| COOPERATING UNITS (if any) University of Vermont School of Medicine, Burlington, Vermont Boys Town, Nebraska National Naval Medical Center, Bethesda, Maryland | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: | PROFESSIONAL: | OTHER: |
| .40 | .20 | .20 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input type="checkbox"/> (c) NEITHER |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Assessments of children's behavior problems, <u>competencies</u> and <u>cognitive functioning</u> are being obtained on normal controls and children with thyroid and sex hormone abnormalities in order to determine whether hormone levels affect patterns of cognitive abilities and behavioral adaptation. The study also tests the hypothesis that increases in particular hormone levels affects patterns of cognitive functioning by improving automatization abilities at the expense of ability to engage in complex <u>perceptual restructuring</u> . It is also designed to determine whether <u>premature craniosynostosis</u> noted in <u>hyper-thyroid children</u> interferes with cognitive functioning. In general, our preliminary analysis shows that girls more than boys exhibit problems of management if they have an endocrine abnormality. This finding suggests a brain and behavior relationship which needs to be investigated further. | | |

Project Description:

Research and theory tend to focus on cognitive disabilities as one substantive area and emotional problems as a separate substantive area. However, from a developmental perspective, cognitive functioning is intimately inter-woven with the social, biological, and emotional aspects of adaptive processes. On the one hand, a child's level of cognitive development is likely to set limits on the types of adaptive strategies available to him or her. On the other hand, social, biological, emotional, and motivational factors are likely to influence the degree to which children make use of their cognitive capacity. The focus of this project is to examine the interface between cognitive functioning and maladaptive behavior.

Work by other investigators has suggested that sex hormone levels affect the action of neurotransmitters in the brain, and that these, in turn, affect the patterning of cognitive abilities. In particular, it has been found that high levels of sex hormones, especially estrogens, are positively correlated with performance on automatization tasks requiring quick, accurate responses, but are negatively correlated with performance on tasks that require complex perceptual restructuring. Our study is designed to test this hypothesis by comparing cognitive functioning and behavioral patterning before and after treatment in children who receive sex hormone supplementation for various endocrine abnormalities. Children with thyroid abnormalities are also being studied because clinical observations have revealed premature craniosynostosis in these children, and it is important to determine whether cognitive and behavioral patterns are affected by this condition.

Measures of cognitive functioning include subtests of the Wechsler Intelligence Scale for Children (WISC), the Embedded Figures Test, Color naming and Object naming test. Differences between perceptual restructuring measures (i.e., WISC Block Design, Embedded Figures Test) and automatization measures (i.e., Color-naming, Object-naming) will be compared at two testing sessions to determine whether changes in hormone levels cause changes in cognitive patterning that do not occur in normal, unmedicated control participants. The child's favorite activities, school performance, and areas of behavioral strength and weaknesses will be examined using interview protocol Form No. ADM512 OMB No. 68. S75110.

At present, our ability to draw conclusions about the effects of sex hormones on childhood disorders is limited due to our small number of participants. However, our analysis, thus far, does suggest sex differences in many of the behaviors of children with endocrine abnormalities. Specifically, girls with endocrine abnormalities do poor school work, keep things to themselves, they prefer to be alone, worry, and are frequently unhappy, sad, or depressed. Children of both sexes appear to have temper tantrums, act stubborn, sullen, and irritable. At present, we have found no behaviors that are more prevalent in males with hormone abnormalities than in females. For the most part, females tend to have more behavior problems than males.

Significance to Biomedical Research:

This study is one of several in the Laboratory in which endocrine dysfunctions in children are viewed in relation to childhood disorders that are cognitive and emotional in nature. It contributes to the body of research knowledge in which behavioral problems and specific biological conditions are being investigated as interrelated processes.

Proposed Course:

We will continue to increase our sample. We plan on expanding our analyses in order to examine other behavior disorders that may be prevalent in children with endocrine abnormalities. During this time we will take a closer look at behavior disorders that vary between and within the sexes.

Publications:

None

| | | | | | | | | |
|---|--|---------------------------------------|--------------------|--------------|----------|-------------------------|---------------------|---------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00257-06 | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | |
| <table border="0"> <tr> <td>PI: Howard A. Moss</td> <td>Guest Worker</td> <td>LDP NIMH</td> </tr> <tr> <td>OTHER: David G. Poplack</td> <td>Senior Investigator</td> <td>POB NCI</td> </tr> </table> | | | PI: Howard A. Moss | Guest Worker | LDP NIMH | OTHER: David G. Poplack | Senior Investigator | POB NCI |
| PI: Howard A. Moss | Guest Worker | LDP NIMH | | | | | | |
| OTHER: David G. Poplack | Senior Investigator | POB NCI | | | | | | |
| COOPERATING UNITS (if any) Various Children's Hospitals throughout the United States National Cancer Institute | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL Staff Years: .30 | PROFESSIONAL: .10 | OTHER: .20 | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The effects of CNS treatment (cranial irradiation and intrathecal chemotherapy) on the <u>cognitive and perceptual functioning</u> of children with leukemia are investigated. One study compares a group of leukemic children in remission with a matched group of siblings on psychological tests (the <u>Wechsler and Bender-Gestalt</u>). These groups are followed-up 12-15 months after the initial assessment. A sample of leukemia patients who have not received CNS treatment and their sibs and a sample of <u>cystic fibrosis</u> patients and their sibs are also being assessed in order to control for effects other than CNS treatment. A second study is prospective. <u>Children in remission from leukemia</u> are evaluated on the Wechsler and Bender-Gestalt. They are tested just prior to <u>prophylactic CNS treatment</u> and one year later to determine possible brain changes resulting in <u>learning disabilities</u> and <u>diminished intellectual functioning</u> . The healthy siblings of the leukemics receiving CNS treatment, who are being studied retrospectively, are used as a control group to check testing effects. A five year longitudinal study investigating the neuropsychological correlates of CNS treatment of leukemic cases at various Children's Hospitals is being <u>undertaken</u> . | | | | | | | | |

Project Description

The chance of survival from childhood leukemia has increased greatly over the past 15 years because of the utilization of new treatment procedures. The main change in treatment that has led to this improved outlook is the inclusion of prophylactic measures consisting of a series of cranial irradiations and intrathecal (spinal) injections of powerful chemical agents, primarily methotrexate.

The purpose of the research is to determine if CNS treatment has deleterious effects on neuropsychological functions and to evaluate the nature and extent of these effects. An additional objective is to determine if an alternative method of treating leukemia, which does not involve direct treatment of the CNS, is equally effective but less neurotoxic. Three studies have been undertaken. One study which has been completed involves the evaluation of intellectual functioning after the completion of CNS treatment (post-treatment study). A second study compares intellectual performance prior to and after CNS treatment. In the third study, the neuropsychological functioning of two groups of leukemia patients are compared; one group receiving traditional CNS treatment, and the other receiving a new form of treatment that is not directly administered to the CNS and possibly is less neurotoxic.

Study I. Post-treatment study.

The sample consists of 34 children who have been in remission for 2 to 5-1/2 years from acute lymphocytic leukemia (ALL), and a sibling control group (mean age = 11 years, with a range of 4 to 23). Thirteen leukemia patients who were treated for their leukemia without the use of CNS treatment and their siblings, and 16 cystic fibrosis patients and their siblings served as control groups. All children were administered a Wechsler Intelligence Test and the Bender-Gestalt.

For most comparisons, siblings performed at a significantly higher level of functioning than did the patients who had received the CNS treatment. The mean Full Scale IQ was 99 for patients and 113 for siblings at an initial testing, and 100 for patients and 116 for siblings at a one year follow-up. There were no IQ differences between the patients and the siblings for the non-CNS treated patient group. The longer the time interval between central nervous system treatment and the psychological assessment the greater is the IQ decrement; and the younger the child at the time treatment was initiated the greater is the apparent IQ loss.

Brighter children were significantly more affected by the CNS treatment than the children of average potential. A measure of variability among Wechsler subtest scores (scatter), which has been used as a diagnostic index of pathology, showed significantly more "scatter" for the CNS-treated patients. From test 1 to the follow-up testing, the "scatter" scores were more stable for the patients whereas the weighted scale scores were more stable for the healthy siblings. Data are being analyzed.

Study II. Prospective Study.

A sample of 45 children with acute lymphocytic leukemia, who receive prophylactic CNS treatment, were administered the same psychological evaluation described in the Post-treatment study. Assessment was made before CNS treatment and one year after treatment. This interval of one year should be sufficient to allow for loss in learning abilities if such losses occur as the result of treatment. The healthy sibling group from the Post-treatment study are used as a control group, to partial out any practice effects that may contribute to the follow-up IQ scores of the patients.

There is sufficient age range in this sample for evaluating whether age at the time of CNS treatment is a factor in contributing to the degree of intellectual deficit. The data collection has been completed and analyses are underway.

Study III. Collaborative Prospective Study Involving Different Medical Treatments.

A large scale clinical study has been initiated under the direction of the National Cancer Institute which includes patients from Children's Hospitals in Seattle, Washington, Northern California (Oakland), Columbus, Ohio, the District of Columbia, and the Pediatric Oncology service of the National Cancer Institute. The purpose is to compare the effectiveness and the degree of neurotoxicity associated with treating acute lymphoblastic leukemia (ALL) patients (a) with cranial irradiation plus intrathecal methotrexate or (b) with high dosage systemic methotrexate infusions. It is known that cranial irradiation and intrathecal methotrexate produce structural changes in the brain and we have also established that this treatment results in lowered intellectual functioning. It is unknown what effects the high dosage infusion methotrexate has on the central nervous system. The sample will consist of children from 1 to 21 years of age who will be evaluated annually for a five to seven year period. The first assessment will be made after remission is brought about from standard chemotherapy but prior to initiation of the central nervous system or high dosage infusion methotrexate treatment. The battery of assessments includes: the Stanford-Binet, McCarthy, or Wechsler scales, in terms of their age appropriateness, for measuring intellectual functioning, a series of neuropsychological procedures for measuring attention, new learning, problem solving, immediate and delayed memory, academic achievement, and sensory and tactile motor functioning (assessments of these specific abilities will be helpful in identifying the localization of brain impairment), the Achenbach Behavior Checklist will be used for measuring social competence and behavioral problems, and a modification of the Block Child Personality Q-Sort procedure (administered to the mothers) to determine if personality characteristics tend to be altered.

This research will include two control groups: a group of matched siblings and a group of children with solid tumors who do not receive any treatment directed at the central nervous system. These control groups will be tested twice at a yearly interval and will be selected so as to be representative of the patient sample in terms of age, ordinal position, and socioeconomic status.

Significance to Biomedical Research:

Medical behavioral interdependencies are the focus of these studies. What may be equally effective treatments of acute lymphocytic leukemia in children may have different consequences in central nervous system damages. Psychological assessments are aimed at evaluating the kind and amount of intellectual impairments involved. Such information is relevant also for planning remedial programs for children who have experienced these treatments.

Proposed Course:

Study I. Data have been analyzed and published. Additional data will be reported in the near future. Some plans are underway to obtain additional data on this sample.

Study II. The data collection has been completed and analyses are underway and will be completed this fiscal year.

Study III. Data collection is in progress and will continue for about four years. Analyses will be initiated in about one year on initial data collected on these subjects and reports will be prepared dealing with performance during earlier stages of the treatment.

Publications:

None

Appendix to Project Number Z01 MH 00257-05

Contract # 263-MD-001195 for \$1480 has been used to reimburse testers, \$40 per case. The tests given are the WPPSI, WISC, WAIS and Bender-Gestalt. The work involved is the equivalent of 1/20 man years.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00265-06 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Chronic Malnutrition and Child Behavior | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Marian Radke-Yarrow | Chief | LDP NIMH |
| Other: David E. Barrett | Research Psychologist | Children's Hosp. Med. Ctr. Boston |
| COOPERATING UNITS (if any) Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .26 | PROFESSIONAL: .01 | OTHER: .25 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Behavioral effects of chronic <u>undernutrition</u> were investigated. Subjects were 138 children, age 6-8, in rural Guatemala who had participated in the INCAP Longitudinal Study. Pre- and postnatal intakes of calorie supplements were the independent variables. Dependent measures were assessments of <u>interpersonal behavior</u> , <u>affect</u> , and coping with environmental requirements. These measures were obtained by observing children in small group activities with peers, and from individual cognitive tests. The results indicate the importance of adequate energy intake in infancy for later behavioral functioning. High <u>calorie supplementation</u> from birth to 2 years was related to higher levels of responsivity, involvement with the environment, and <u>emotional expressivity</u> as well as to moderate activity level, and low levels of passivity. Except for <u>attentional measures</u> , <u>cognitive measures</u> , were not strongly predicted by supplement intake. | | |

Project Description:

The effects of early chronic malnutrition on children's behavioral competence at school-age are investigated. Two areas of past research provide a theoretical base: Research on animals with a history of undernutrition identifies a consistent pattern of behavioral abnormalities that includes apathy, passivity, attentional impairments, reduced exploration, avoidance of new stimuli, and abnormal responses to social initiations. Malnourished human infants show similar behavioral disturbances. Such behaviors, if they continued, would impair the organism's social interactions and affect and interfere with competency.

In the present study, relations between pre- and postnatal nutrient intake and behavioral functioning at ages 6 to 8 were examined. Subjects were 138 children from three villages in eastern Guatemala. All had participated in the Institute of Nutrition of Central America and Panama (INCAP) Longitudinal Study (1969-77) in which ad libitum calorie supplements had been provided to pregnant women and later to their children. Supplement caloric intake measures were the independent variables: (a) mother supplement calories during pregnancy, (b) child supplement calories from birth to 2 years, and (c) child supplement calories from 2 to 4 years. To ensure that supplement effects were not confounded with pre-existing village differences, analyses were done within and across villages. A composite measure of socio-economic level of family was included as a covariate in the analyses. Assessments of behavioral functioning were made from observations and standard tests. Children were observed in two 2 1/2 hour sessions in six-person groups of peers, and in contacts with adults. Sessions were constructed to involve free play, problem solving, competitive activities, and need for impulse control. Detailed behavioral records were made. Cognitive assessments were made from a battery of standard tests.

Findings are supportive of the hypothesis that undernutrition in early life may adversely influence behavioral functioning in childhood. Maternal caloric supplementation during pregnancy and child supplementation during the first two years were related to greater interest in the environment, more social responsiveness, higher levels of interactive involvement, more expression of affect, and moderate activity level at school age. Attentional characteristics were also related to malnutrition, although not as strongly as were the social-emotional variables. Differences were found on tests measuring ability to attend to directions, persist on a difficult task, and attend to detailed stimulus configurations. In contrast to relations between supplementation and behavior, relations of height and weight to behavior were inconsistent. Findings were similar for boys and girls. In general, within-village findings support the general findings.

Significance to Biomedical Research:

Early nutritional deficits have for a long time been studied in relation to effects on children's intelligence to cognitive skills. The importance of these studies (MH 02138-04 LDP and MH 00265-06 LDP) lies in extending investigation to behavioral disorders that are attentional, emotional, and

interpersonal in nature. These studies help to increase the awareness of a broad range of medical-behavioral interrelation.

Proposed Course:

This is a final report.

Publications:

Barrett, D.E. and Radke-Yarrow, M.R. Effects of early caloric supplementation on social-emotional functioning at school-age. Developmental Psychology, 1982, 18(4).

Barrett, D.E. and Radke-Yarrow, M.R. Effects of early caloric supplementation on social-emotional functioning at school-age. In J. Brozek and T. Massaro (Eds.), Benchmark papers in behavior. Stroudsburg, Calif: Hutchinson-Ross Publishers, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02132-05 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Children's Misdeeds, Parental Discipline, and Children's Compliance | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Carolyn Zahn-Waxler Research Psychologist LDP NIMH | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .25 | PROFESSIONAL: .05 | OTHER: .20 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The interaction of children's <u>misbehaviors</u> and parents' <u>disciplinary methods</u> , and their effects on children's <u>compliance</u> and <u>internalization of rules</u> were studied. Twenty-four mothers of one-to-two year old children were trained to report incidents of children's negative emotional interactions, from which sequences of children's misbehaviors, maternal discipline, and children's subsequent compliance or noncompliance were coded. Specific forms of misbehavior from these young children influenced the forms of discipline received; children's harms against persons were associated with psychological discipline methods such as reasoning and guilt induction. Destruction of property and lapses in self control were linked with techniques of physical punishment and love withdrawal. Love withdrawal was the most effective parental technique in securing compliance from the child at least in the immediate situation. | | |

Project Description

Recent conceptualizations of children's affective development have stressed the contribution of both parents and children in influencing normal and deviant child development. The purpose of this study is to investigate their joint contributions to children's development by examining the interactions among children's misbehaviors, parental discipline practices, and children's compliance or non-compliance to established familial and societal norms. Do children influence the forms of discipline they receive? Do different discipline practices particularly physical punishment and love withdrawal have different effects on children's compliance vs. noncompliance?

Twenty-four middle class mothers and their children were studied for nine months when the children were one-to-two years old. Mothers trained in observational techniques reported incidents of children's negative emotional encounters with other persons. From these reports, sequences of children's misbehaviors, mothers' discipline practices, and children's compliance or noncompliance were obtained.

By the second year of life children engaged in behaviors that elicited a range of physical, verbal, and psychological interventions from their caregivers. Often multiple forms of discipline were used within a given incident. The forms of discipline were determined, in part, by the particular ways in which children misbehaved. Children's aggressions against persons (e.g., biting, hitting) often were linked with psychological methods of discipline such as reasoning and highlighting the painful consequences for others of the child's aggressive actions. The latter maternal behavior, when expressed frequently and with intensity, may be a form of guilt induction. Misbehaviors involving damage to objects and loss of self-control often were associated with power assertive discipline such as physical punishment and love withdrawal. Love withdrawal is not used frequently; it tends to be used after other methods have failed and it is particularly effective in obtaining immediate compliance from the child. There are reasons for assuming that its long-term consequences may be more complex.

Significance to Biomedical Research

There is strong evidence to indicate that severe emotional problems and aggression in later childhood often can be traced to early disturbances in parent-child interaction. These problems can result from deviant parental practices used to control children and from inherent temperament differences in children's responses to discipline. Identification of these factors facilitates planning early preventive strategies and therapeutic interventions in maladaptive parent-child interactions.

Proposed Course

This is a final report.

Publications:

Zahn-Waxler, C. and Chapman, M. Immediate antecedents of caretakers' methods of discipline. Child Psychiatry Hum. Dev., in press.

Chapman, M. and Zahn-Waxler, C. Young children's compliance and noncompliance to parental discipline in a natural setting. Int. J. Beh. Dev., in press.

Zahn-Waxler, C. and Chapman, M. The effects of children's transgressions on parents' methods of discipline. ED 184 729. Eric Reports, Educational Resources Information Center, Arlington, Virginia.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02135-05 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Emotional Development in Children of Bipolar Depressed and Normal Parents | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Carolyn Zahn-Waxler | Research Psychologist LDP NIMH |
| OTHER: | Marian Radke-Yarrow Mark Cummings Ronald Iannotti Leon Cytryn Donald McKnew Yolande Davenport | Chief Staff Fellow Guest Worker Research Psychiatrist Research Psychiatrist Social Worker LDP NIMH LDP NIMH LDP NIMH BPB, LDP NIMH BPB, LDP NIMH CPB NIMH |
| COOPERATING UNITS (if any) Laboratory of Biological Psychiatry, NIMH Clinical Psychobiology Branch, NIMH | | |
| LAB/BRANCH Laboratory of Developmental Psychology, NIMH | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .60 | PROFESSIONAL: .40 | OTHER: .20 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This research is concerned with the <u>psychological development</u> of young children from families in which one parent has a history of <u>bipolar affective illness</u> and children from families in which there is no diagnosed parental psychopathology. Children are studied longitudinally beginning at 12 months of age. Observations in the home and mothers' reports provide data on day-to-day functioning. In a series of laboratory sessions, assessments are made of children's affective and interpersonal functioning with peers, mothers, and unfamiliar adults. Children from bipolar families were particularly likely to have <u>behavior problems</u> , <u>disturbances in attachment patterns</u> , deficits in social-cognitive functioning, and difficulties in interaction with peers. Child-rearing practices were also different in normal and bipolar families. | | |

Project Description:

The focus of this research is on the psychological development of children from normal families and children from families in which one parent has a pervasive and enduring affective disturbance (bipolar affective disorder). The familial recurrence of manic-depressive illness across generations is well documented, evidence for a genetic predisposition to the disorder. The affective environments in which children of manic-depressive parents are reared may also play a significant role in the transmission process. Several environmental factors may place the children at risk: the high levels of stress and distress that characterize manic-depressive families, the unpredictability of parental affect and behavior, difficulties in establishing good interpersonal relationships, and possible atypical child-rearing practices in these families all constitute affective and behavioral events that may deviate from those experienced in normal family environments.

Seven children with a bipolar parent (3 fathers, 4 mothers) and 21 control children from normal families were studied (all were SADS screened). A subsample of the control group were children whose parents had been screened and selected on the basis of total absence of psychopathology in the immediate family. Children entered the study at one year of age and were studied intensively for two years. Data were obtained from multiple sources in naturalistic and experimental settings. (1) Mothers were trained to report on children's responses to positive and negative emotions. (2) Children's reactions to simulations of distress emotions (e.g., sorrow, depression, pain) were videotaped during home visits. (3) Cognitive skills (object permanence and self-other differentiation) were tested in the home in the second year of life. (4) Between 24 and 30 months children's interactions with peers, mothers, and adult strangers were examined in the laboratory. Manipulations were introduced in which the background environment was experimentally varied in positive and negative affect. (5) Throughout the study, home visitors obtained current data on child behavior problems (e.g., fears, sleep disturbances, eating problems). (6) Parental rearing practices and discipline techniques were also assessed from structured tests (e.g., Block Q-sort) and from direct observation and rating procedures.

Children of bipolar patients as a group differed from the group of children from normal families in the following ways: They had significantly more frequent and more severe behavior problems based on a composite rating of symptoms than children from normal families (symptoms include phobias, sleep disturbances, eating problems, social withdrawal, high activity, and self-punitive behaviors such as head-banging). Disturbances in their patterns of attachment to the mother were identified in several assessments spanning a period of a year. Their social-cognitive skills were similarly disrupted. There were early disturbances in their interactions outside the family setting. While bipolar patients' children did not differ from children from normal families in overall levels of aggression, their aggression was more maladaptive; for example, they showed more undirected aggression (i.e., not leading to conflict resolution) while interacting with peers. Their relative inability to tolerate frustration in a structured task was reflected in significantly more hostility toward the adult present. As a group, children from bipolar

families were significantly less altruistic toward their peers than children from normal families, particularly with regard to their ability to share. Children from bipolar families also tended to show more extreme reactions to their parents' distress, e.g., either being extremely solicitous and compassionate (assuming a caregiver role), or showing extreme avoidance of, and withdrawal from, the distress. In the laboratory setting, children from bipolar families were more sensitive to manipulations of the affective environment. In simulated anger between experimenters (intended to be overheard by pairs of children as they played together), the social interactions of children from bipolar families relative to control were considerably more disrupted. Thus, although positive coping skills and attributes were present a surprisingly large number of symptoms and problems surfaced in young children from bipolar families.

The affective rearing environments of these children were also deviant. Mothers in bipolar families reported feeling significantly more anger, fear, and sadness than mothers from normal families, and were rated by home visitors as more disorganized, less active, more unhappy, more ineffective, more tense, and more inconsistent in their caregiving practices. On the Block Q-sort, mothers from bipolar families emerged as different from normals on a number of affective dimensions: they were less likely to encourage the child to be open to new experiences, more likely to feel negative affect toward the child, less likely themselves to be open in their expression of affect toward the child, and more likely to be overprotective. The consistent prevalence of such patterns and practices could be one set of factors that places children of manic-depressive parents at risk for emotional and interpersonal problems.

Significance to Biomedical Research

Manic-depressive illness is believed to be a genetic disorder. Transmission of the illness across generations, however, may also be influenced by the disordered emotions and environments in which children of manic-depressives are reared. This research identifies some of the deviant child-rearing practices used in bipolar families and the emotional problems that may result for the children as early as the first years of life. This information is directly relevant in planning prevention and intervention strategies in families with severe emotional problems: the aim is to decrease the likelihood that future generations of children will be affected by the affective disorders of their parents.

Proposed Course

Manuscripts reporting detailed findings are in preparation and will be completed in the coming year. Longitudinal follow-up into early childhood of these families is planned. Biological, behavioral, and affective indices of child functioning will be obtained.

Publications:

Zahn-Waxler, C.: Maternal child rearing practices in relation to children's altruism and conscience development. In Hare, A. P., Blumberg, H. H., Kent, V. and Davies, M. (Eds.), Small Groups: Social Psychological Processes, Social Action and Living Together. London, John Wiley & Sons, 1982, in press.

Project Description:

The effects of nutritional deficit at different periods in early life on the child's social-emotional behavior at school age were investigated. Cognitive abilities and sensorimotor skills were also assessed. Research on malnourished animals identifies a critical complex of behavioral abnormalities which includes apathy, inability to sustain attention, reduced exploration and curiosity, fearful avoidance of new stimuli, failure to respond normally to social initiations. This background of research provided the theoretical framework for the present study.

The children in this study are 65 seven-year-olds, from Anglo families, living in poor urban areas of Southern California. All families are below a poverty income level.

Information about nutritional histories and current health was obtained from a mother interview, anthropometry, and hemoglobin analyses. Children were assigned scores on five composite variables, each representing risk for developmental impairment due to nutritional stress at a different phase of development. The composites were: conditions of pregnancy, neonate health, child health from birth to two years, child health from two years to present age, and current anthropometry and hemoglobin. To control for SES factors in the data analyses, data on conditions of housing, parents' occupation and mother's education were obtained.

Children were observed in 6-person play groups for a total of 5 hours. Natural and experimental situations were involved. Observational records included: behavior with peers, behavior with adults, interaction with the environment, behavior alone, activity level, and emotional expression. Individual cognitive and sensorimotor tests were administered.

Children whose mothers had been undernourished during pregnancy showed lower levels of affiliative and positive interactions with peers than children whose mothers had not been undernourished during pregnancy. They were also more dependent, more often sad in affect, and less interested or involved. Prenatal undernutrition was not related to intellectual test performance at early school age. Children who were undernourished during preschool to early school years tested lower on tests of sensorimotor functioning, intelligence and achievement.

Significance to Biomedical Research:

Early nutritional deficits have for a long time been studied in relation to effects on children's intelligence to cognitive skills. The importance of these studies (MH 02138-04 LDP and MH 00265-06 LDP) lies in extending investigation to behavioral disorders that are attentional, emotional, and interpersonal in nature. These studies help to increase the awareness of a broad range of medical-behavioral interrelation.

Proposed Course:

This is a final report.

Publications:

Barrett, D.E. An approach to the conceptualization and assessment of social-emotional functioning in studying nutrition-behavior relationships. American Journal of Clinical Nutrition, 1982, 35.

Barrett, D.E. Measurement of children's social-emotional behavior. In J. Brozek and B. Schurch (Eds.), Critical evaluation of key issues in the research on malnutrition and behavior. Berne, Switzerland: Hans Huber, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02139-04 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Children's Behavior in Situations of Emotional Distress | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Carolyn Zahn-Waxler OTHER: Michael Chapman Ronald Iannotti | Research Psychologist Research Psychologist Guest Worker | LDP NIMH Max Planck Inst. West Germany LDP NIMH |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .27 | PROFESSIONAL: .07 | OTHER: .20 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Cognitive and affective mediators of altruism</u> were investigated from a developmental perspective. Relations among children's social-inferential abilities, emotional arousal, and behavioral interventions in others' circumstances of distress were examined. <u>Emotional arousal</u> and <u>prosocial interventions</u> were measured in response to simulations of emotion. Assessments of <u>perspective taking</u> , <u>role taking</u> and <u>prosocial moral reasoning</u> were also made. Sixty children ages four to eleven were studied. Social-cognitive abilities and prosocial interventions frequently increased with age while levels of emotional arousal did not change with age. Controlling for age, both intelligence and affective arousal were predictors of prosocial interventions. | | |

Project Description

Cognitive understanding of, and affective response to, another's experiences are investigated as mediators of altruistic behavior,--action taken to aid or alleviate distress in another person. The ability to put one's self in the place of another and understand that person's internal state (perspective taking or role-taking) is assumed to provide a necessary knowledge base for action. Affectively experiencing what the other is experiencing (empathy), may provide the motivation to act. The relationships among cognitive, affective, and action variables were examined.

A cross-sectional sample of five girls and five boys at 4, 5, 6, 7, 10, and 12 years of age has been studied in the laboratory. Children's cognitive abilities were measured on a series of tests: the Peabody Picture Vocabulary Test, forward and backward digit span, an emotion identification test, and perspective-taking tests of Flavell and Chandler. Tests of prosocial moral reasoning consisted of children's interpretations of eight stories involving distress incidents of kinds common to children's own experiences. Simulations of emotion (e.g., an injured adult, a crying baby) were used as the stimuli for arousal of affect and for possible prosocial interventions.

Social-cognitive abilities and prosocial interventions frequently increased with age, and prosocial moral reasoning abilities sometimes changed with age, while levels of emotional arousal were similar in children of different ages. Controlling for age, intelligence test scores, prosocial moral reasoning measures, and affective arousal were only moderate predictors of altruistic interventions. Measures of perspective-taking were not predictors. Prediction patterns sometimes differed depending upon whether the recipient of altruistic behavior was an adult or infant. Attributions or projections of guilt in the prosocial moral reasoning test were positive predictors of helping behavior toward adults while themes of prosocial activity on reasoning tests were related to altruistic behaviors toward infants.

Significance to Biomedical Research

This research identifies some of the characteristics of children that help to determine their capacities for dealing adaptively with stressful situations, particularly emotionally arousing stimuli in their interpersonal environments. Therapeutic intervention strategies may be planned more effectively by taking such information into account.

Proposed Course:

This is a final report.

Publications:

Zahn-Waxler, C., Iannotti, R., and Chapman, M. Peers and prosocial development. In Rubin, K. and Ross, H. (Eds.), Peer Relationships and Social Skills in Childhood, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02140-03 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Interrelations of Early Cognitive Development and Empathic Behavior | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Michael Chapman Staff Fellow LDP NIMH OTHER: Carolyn Z. Waxler Research Psychologist LDP NIMH Marian R. Yarrow Chief LDP NIMH | | |
| COOPERATING UNITS (if any) NONE | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .00 | PROFESSIONAL: .00 | OTHER: .00 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The Principal Investigator on this project is not currently employed in the Laboratory. Data already collected from analyses in this project will be incorporated in Z01 MH 02139-04. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: right;">201 MH 02142-04 LDP</div> |
| PERIOD COVERED <div style="text-align: center;">October 1, 1981 through September 30, 1982</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Behavioral Studies of Children with Juvenile Diabetes</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Howard A. Moss | Guest Worker | LDP NIMH |
| OTHER: Blanche S. Jacobs | Guest Worker | LDP NIMH |
| COOPERATING UNITS (if any) <div style="text-align: center;">Local Community Physicians in Private Practice</div> | | |
| LAB/BRANCH <div style="text-align: center;">Laboratory of Developmental Psychology</div> | | |
| SECTION | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</div> | | |
| TOTAL Staff Years: <div style="text-align: center;">.40</div> | PROFESSIONAL: <div style="text-align: center;">.10</div> | OTHER: <div style="text-align: center;">.30</div> |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The objective of this research is to identify attitudes, concerns, and response patterns that children and their parents exhibit in relation to the child's having <u>juvenile onset diabetes</u> for a period of years. The main focus of the research is identifying stabilized behavioral or adaptive patterns that have evolved as reactions to the disease and to ascertain the factors that have led to a particular style of adaptation. Particular attention is given to whether a child develops a <u>passive-helpless orientation</u> or attempts to <u>master</u> and/or <u>excel</u> in some area of endeavor as a life style in coping with a chronic illness. Children between 11 and 18 years of age who have had juvenile onset diabetes for three years or longer are studied. A matched control group of healthy children is also seen. Data consist of interviews, questionnaires, and standardized tests administered to the children and their mothers and life event data obtained from school and medical records. This research is relevant to issues of how children in general cope with stress. </p> | | |

Project Description:

Chronic and life threatening illness in childhood presents special challenges, conflicts, and stresses for the stricken child as well as for his/her family. The purpose of the research is to identify response patterns that children and their parents develop as a function of the child's being ill with juvenile onset diabetes. A central hypothesis is that the behavior of children who are handicapped by chronic adversity will be polarized toward exhibiting extremes of helplessness or heightened mastery behavior. Children who are living with a personal misfortune will tend to be bimodally distributed whereas healthy or unaffected children will be normally distributed on this dimension.

The research involves the study of 25 children between 11 and 18 years of age who have had juvenile onset diabetes for three or more years. It is assumed that in that period of time stabilized behavioral reactions to the illness will be manifested. A control group of children with no history of chronic illness or permanent handicaps also were studied. The children and their mothers are seen for a session lasting from 2-1/2 to 3 hours. This session consists of an interview with the child, a separate interview with the mother, and the administration of standardized psychological tests aimed at identifying personality characteristics, child rearing practices, goal setting behavior, and standards of competence. The interview with the child and the mother focuses on the history, affective reaction, and adaptation to the illness and with the fostering and acquisition of coping styles, standards, and evaluating helplessness and mastery orientations in the child. For the control group, the interview and the psychological tests were administered.

Significance to Biomedical Research:

This study focuses on the psychological concomitants or consequences of chronic physical illness in children. The findings although about juvenile diabetes have generalizable applicability to other disease states.

Proposed Course:

Data collection has been completed. Analyses are underway and should be completed this fiscal year.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER ZOI MH 02143-04 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Psychosocial and Biomedical Interactions in Juvenile Diabetes | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Beatrix A. Hamburg Other: Gale Inoff Michael H. Ebert Irwin J. Kopin Phillip Gorden David Newsome | Guest Worker Research Psychologist Clinical Director Chief, Lab. of Clin. Science Chief, Clinical & Cellular Biology Section Chief, Retinal & Ocular Conn. Tissue Diseases Section | Children's Hosp. Med. Center, Boston LDP NIMH NIMH LCS NIMH DB NIAMDD CB NEI |
| COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH Diabetes Branch, NIAMDD Clinical Branch, NEI | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .10 | PROFESSIONAL: .00 | OTHER: .10 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Stress</u> influences all aspects of <u>health status</u> and <u>behavioral adaptation</u> . However, although <u>emotional stress</u> and <u>psychosocial factors</u> have all been thought to be influential in the course and onset of <u>insulin-dependent diabetes</u> , there has been little systematic study of the syndrome in terms of <u>biomedical and psychosocial interactions</u> . This project sought to broaden the understanding of the interplay of biomedical and psychosocial factors by (1) developing a <u>conceptual model</u> for clinical care and research studies involving stress-related processes in physical illness and (2) systematically examining biomedical and psychosocial interactions in the context of a <u>camp for diabetic children</u> . One set of findings involved degree of <u>diabetic control</u> (as measured by <u>glucose levels</u> in the urine) and <u>knowledge of diabetes</u> ; results indicated that acquisition of high levels of knowledge of diabetes may reflect a coping effort in response to the stress of poor diabetic control. Regarding the relationship between diabetic control and degree of <u>feeling in control</u> over one's life events, sex differences were found and <u>interpreted</u> as reflecting potential differences between the sexes in their responses to stress which were actualized by the <u>stress of poor diabetic control</u> . | | |

Project Description

Stress influences all aspects of health status and behavioral adaptation. This project sought to broaden the understanding of the interplay of biomedical and psychosocial factors by (1) developing a conceptual model for clinical care and research studies involving stress-related processes in physical illness and (2) systematically examining biomedical and psychosocial interactions in the context of a camp for diabetic children.

It has become increasingly recognized that medical outcomes in chronic illness depend substantially on behavioral and psychosocial factors as well as biomedical factors. Recognition also is now being given to the importance of studying behavioral variables as significant outcomes, in their own right, in the course of living with chronic illness. Two hundred and twelve boys and girls, age 5-19 years, were studied during two-week sessions at a camp for insulin-dependent children. Our main research questions involved relationships between degree of diabetic control and two behavioral variables expected to influence or be influenced by degree of diabetic control. They are: knowledge of diabetes and locus of control. Knowledge of diabetes was chosen as a variable for several reasons. First, diabetes management and self-care require considerable mastery of information and technical skill, but research has shown that, for many people with diabetes, knowledge and skills are inadequate. Secondly, information-seeking is a coping strategy often employed by people attempting to gain control over a chronic illness. For these reasons, measurement of knowledge of diabetes was viewed as an assessment of the degree to which the individual attempted to equip himself/herself to manage or control diabetes.

The second behavioral variable, locus of control, represents the degree to which the person feels able to control the events and circumstances in his/her life. Because diabetes is a disorder in which there is an ongoing struggle to control the illness without, at the same time, letting it control one's life, this variable seemed especially appropriate. Furthermore, diabetes is an illness in which there is special need to take personal responsibility for influencing one's health and life events. In addition to possessing knowledge and technical skills, the diabetic person must believe or expect that his/her actions have potential for improving health outcomes. The locus of control measure indexes this expectancy dimension. Relationships involving other medical history, behavioral, and demographic variables describing the children also were examined.

In general, diabetic control, as measured by counselor-monitored urine tests made across a two-week period, improved across age groupings. This is especially interesting because it is generally believed that diabetic control worsens during adolescence.

We also found that children's knowledge of diabetes increased with age for both boys and girls; additionally, girls scored higher than did boys. For both sexes (but especially for boys), diabetic control was negatively related to knowledge of diabetes; that is, the worse the diabetic control, the higher the degree of knowledge. Acquisition of an unusually high level of knowledge was interpreted as a coping effort in response to the stress of poor diabetic control. (Effects of age and duration of diabetes are statistically controlled in the relationships with diabetic control.)

Regarding locus of control, both boys and girls became more internal (feeling in control) with age; also, girls were more internal than were boys. Locus of control also was related to diabetic control, but the relationship was different for boys and girls. Boys in poor diabetic control tended to be more internal in locus of control (feeling in control, ready to take action to confront their difficulties), and girls in poor diabetic control tended to be more external (feeling powerless, acting compliant). This sex difference was interpreted as reflecting sex differences in responses to stress. While this issue is complicated and is widely discussed, there is evidence that suggests that males and females sometimes show different response patterns to stress. For example, in other investigations, sex differences have been found in temperament and in prevalence of various symptoms of psychopathology. These sex differences may be summarized in terms of a bipolar dimension of response to stress with increases in activity for males at one pole and withdrawal for females at the other.

In our data, it was viewed that poor diabetic control is stressful and, if the typical male response to stress is to become more active (which could translate to internal on locus of control), one would expect that the male response to poor diabetic control would be to become more internal on locus of control. For females, poor diabetic control also is stressful and, if the typical female response to stress is to show withdrawal behaviors (which could translate to external on locus of control), one would expect that the female response to poor diabetic control would be to become more external on locus of control. Thus, the stress of poor diabetic control may actualize potential sex differences in responses to stress which may be observed in behaviors conceptualized as locus of control.

Medical history data were available and included parental report of items regarding emotional or behavior problems in the child. Sixteen percent of the children were described by parents as having some degree of emotional or behavior problem (e.g., depressed, low self-esteem, ran away, fearful, dependent). Boys and girls were very similar. The age group having the highest prevalence was the 9-11 year olds. This age range coincides with the age during which there typically is parent and physician pressure toward assumption of greater responsibility regarding management of diabetes (e.g., giving own injections, managing the diet).

Symptoms of hypoglycemia exhibited by each child also were available in the medical history records, and these data also were analyzed. It should be noted that while there is implicit recognition that hypoglycemia is evidenced in discrete symptom complexes that are specific for a given child but vary across children, very little attention has been given to this aspect of diabetes. Our initial attempts to provide descriptive data regarding this area of diabetes indicate that this should be a fruitful area for further study. We also found that 31% of the children had mood or behavior change as one of their symptoms of hypoglycemia; it is thought-provoking that effects of low blood sugar do not carry over into mood and behavior to the same degree for all people. Further investigation of glucose metabolism as well as response to stress may clarify some of the origins of the affect and behavioral variability we label as moodiness, irritability, and unpredictability in non-diabetic as well as diabetic people.

Manuscripts describing the conceptual model and the camp study have been accepted for publication. This is a final report.

Significance to Biomedical Research

This research has significance for biomedical research in that it examines interrelationships between stress, psychosocial factors, and health status in children and adolescents with insulin-dependent diabetes. Diabetes is especially appropriate for the study of biomedical and psychosocial interactions because diabetic control (i.e., glucose regulation) is known to be affected by stress. Additionally, diabetes requires extensive self-care for management of the illness, and stress affects the nature of the behavioral response to having a chronic illness. This project also has involved the development of a conceptual model for clinical care and research involving stress-related processes in psychosomatic and other physical illness.

Proposed Course

The project is terminated.

Publications:

Hamburg, E. A. and Inoff, G. E. Coping with predictable crises of diabetes. Diabetes Forecast, in press.

Hamburg, E. A. and Inoff, G. E. Relationships between behavioral factors and diabetic control in children and adolescents: A camp study. Psychosomatic Medicine, in press.

Hamburg, E. A. and Inoff, G. E. A multivariate biomedical-psychosocial model of stress and health outcomes (using adolescents with diabetes as a paradigm). J. of Adolescent Health Care, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02144-02 LDP | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Studies of Child Rearing in Normal Families and in Families with Pathology: A Research Paradigm | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Marian Radke-Yarrow</td> <td style="width: 20%;">Chief</td> <td style="width: 40%;">LDP NIMH</td> </tr> <tr> <td>Carolyn Zahn-Waxler</td> <td>Research Psychologist</td> <td>LDP NIMH</td> </tr> <tr> <td>Leon Kuczynski</td> <td>Foreign Visiting Fellow</td> <td>LDP NIMH</td> </tr> </table> | | | PI: Marian Radke-Yarrow | Chief | LDP NIMH | Carolyn Zahn-Waxler | Research Psychologist | LDP NIMH | Leon Kuczynski | Foreign Visiting Fellow | LDP NIMH |
| PI: Marian Radke-Yarrow | Chief | LDP NIMH | | | | | | | | | |
| Carolyn Zahn-Waxler | Research Psychologist | LDP NIMH | | | | | | | | | |
| Leon Kuczynski | Foreign Visiting Fellow | LDP NIMH | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | | | | |
| SECTION | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | |
| TOTAL Staff Years: 4.75 | PROFESSIONAL: .75 | OTHER: 4.00 | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The objective of this research is the development of a research strategy (a) by which <u>parental rearing</u> functions and modes of behaving in the parent role are kept intact but (b) by which experimental control over selected functions or events is also exercised. Mothers and children are observed over a series of half-days, in an informal laboratory (apartment). Conditions allow and encourage daily routines and <u>natural</u> behaviors to take place (eating, playing, resting, disciplining, watching TV, etc). There is also an underlying structure in which certain standard events are introduced to bring out given classes of response. Microanalyses and broader interpretive analyses are geared to identifying significant aspects of rearing that become visible in this direct observation of rearing processes. In this project a major focus is on the conceptualization and measurement of rearing. This research paradigm is the basis of a series of investigations on specific aspects of rearing (<u>affective communications and control techniques</u>), Projects MH 02152 and MH 02155, and rearing processes in families selected in terms of criteria of <u>psychological disorders</u> and of <u>normality</u> (MH 02156). | | | | | | | | | | | |

Project Description:

In most theories, the environment in which a child is reared is considered a major contributor to the child's development. Conceptualization of rearing dimensions and variables, accessibility of the phenomena to be studied, and techniques of assessment present enormous problems for research. In this research, our focus is on parent behavior. Whatever the built-in developmental stage or capacities of the child, the environment provided by the parent(s) significantly tunes the child's behavior. The elements of rearing that are critical in the child's emotional-social development are not well studied, especially past the infant period. Existing conceptualizations and methods for assessing parental behavior are in our opinion, inadequate. We have given much effort, therefore, to (a) developing conceptualizations of rearing that break away from the constricted views of rearing as "control techniques" and (b) developing a research paradigm that gives direct and prolonged access to maternal rearing behavior and to child behavior. The research approach that has been developed is one in which parental rearing functions and modes of behaving in the parent role are kept intact, but in which experimental control over selected functions or events is also exercised.

Mothers and their young children are observed with each other, over extended periods of time, under conditions that maximize the advantages of naturalistic observation and realize as well some of the control of laboratory conditions. Mothers and children are seen in a laboratory apartment, an informal home-like setting, for a series of half-days. Conditions and facilities allow and encourage usual daily routines, demands, and interactions to take place. Each day has, however, an underlying controlled structure in which certain standard events occur that present specific challenges. It is possible to observe, for example, how attachment patterns are played out in rearing, what anticipatory and reactive behaviors are used by mothers, what stable or unstable expectancies develop in the parent-child relationship, what emotions occur and how they are regulated. Home observations, standard paper and pencil assessments and interviews are also used as data sources. Families are seen at three time intervals over a period of three years.

Parent and child behaviors are coded "live" and from audiovideo records. Multiple levels, but complementary systems, of coding are utilized: molecular frequency counts and sequential analyses, and behavior units involving higher levels of organization and interpretive categories. Specific rearing functioning (e.g., regulating, teaching, monitoring, playing, providing physical care, etc) are retained in the analyses, in order to examine behaviors of mother and child in relation to specific contexts of interaction. Indirect maternal influences (energy level, affective state, etc.) and child-directed influences (e.g., disciplining the child, showing affection, punishing) are examined. The cognitive, affective, and interactive contents of rearing are investigated.

Approximately 40 families have been studied. A larger sample will be obtained. Follow-up visits of the families are getting underway.

From preliminary analyses of the maternal behaviors, a number of variables emerge that distinguish rearing environments. They are: (a) various rearing activities (care, discipling, teaching) receive very different weightings in different families; (b) the behavior settings provided by the parent in different families vary greatly in their complexity; (c) the amount and quality of time spent with the child varies; (d) environments vary in mothers' monitoring of and interest in the child; (e) amount and content of both mothers' incidental and purposeful teaching show wide contracts. These dimensions have generally not been assessed in research on rearing influences.

Potential biasing influences of different research strategies can be assessed in this study. For example, in home observational studies of child development, mealtime or problem-solving have conventionally been used as "standard" settings in which to measure family interaction. In such observations, certain requirements are generally imposed on the families, such as everyone's presence at the table or everyone's engagement in the problem solving. These constraints mask the extraordinary variation in behavior that occurs across families when, as in the present design, mother and child impose their own imprint on these activities.

This research paradigm is the basis of a series of investigations in which specific aspects of rearing (affective communications, control techniques) (Projects Z01 MH 02152, Z01 MH 02155) are studied, and in which families selected on criteria of psychological disorders and normality (Z01 MH 02156) are investigated.

Significance to Biomedical Research:

In order to conduct research on family environmental contributions to children's development, methods of sensitively measuring the environment are needed. This aspect of measurement has been seriously deficient in research on environmental contributions to children's behavior problems and psychiatric disorders. In this study, an effective research method has been developed. (See MH 02156-03 LDP for the substantive content of this research.)

Proposed Course:

This is a longitudinal study in which families are followed over a period of 3 years. The sample base which is now about 40 will be doubled. It is anticipated that 4 to 5 years will be required for completion of data collection.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02145-03 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Impact of Anger on Emotional Disturbance and Aggression in Children | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | E. Mark Cummings | Staff Fellow |
| | | LDP NIMH |
| Other: | Carolyn Zahn-Waxler Marian Radke-Yarrow | Research Psychologist Chief |
| | | LDP NIMH LDP NIMH |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: | PROFESSIONAL: | OTHER: |
| .36 | .26 | .10 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> The impact of others' emotional interactions on young children was assessed in a programmatic series of home and laboratory studies. Others' angry interactions in the environment of the child, but not directed to the child, frequently produced overt distress in both preschoolers and school-age children. Patterns of children's emotional responding to others' <u>anger</u> tended to be stable across time. Frequent exposure to <u>interparent hostility</u> resulted in increased visible disturbance in the child. Both preschool and school-age children most often responded to others' <u>affection</u> with smiling, laughing and/or attempts to participate in affectionate interactions. A series of laboratory studies involving two-year old children demonstrated that exposure to anger between adults tended to increase the intensity of later aggression towards peers. Observations of affiliative or prosocial interactions between adults reduced the frequency and intensity of the child's aggression toward peers. In contrast, others' angry and prosocial interactions had little effect on <u>altruism</u> towards peers. This research indicates the need to consider the ambient emotional climate in the home as a factor in early emotional development. </p> | | |

Project Description:

Research on the factors influencing early emotional development has focused on direct interactions between children and others. However, children may also be significantly affected as bystanders to others' emotional behavior, e.g., conflicts between the parents. A programmatic series of studies was conducted to assess the impact of others' emotional interactions on young children.

One investigation focused on the emotional responses of 1- to 2-1/2 year old children to real angry and affectionate interactions in naturalistic settings. Mothers trained as observers provided narrative reports of incidents in which their children were exposed to angry or affectionate interactions between family members in the home. Others' anger tended to act as environmental stresses. The most common emotional response to anger was distress, which was particularly likely as a reaction to conflicts which included physical attack. Children's individual patterns of responding to anger tended to be consistent across situations. However, there was evidence that exposure to anger between the parents had a cumulative effect: Children most often witnessing parental conflicts were most likely to evidence emotional disturbance in response. This suggests that temperamental dispositions evident in the face of others' emotions may have their origins in children's experiential history as well as in possible biologically-based tendencies. The responses of children as bystanders to others' affection were considerably more positive. Smiling, laughing and/or attempts to participate in affectionate interactions were the most common reactions, although jealousy was sometimes a factor in otherwise positive patterns of responding.

A series of laboratory studies was conducted to examine the impact of exposure to angry or affectionate interactions between adults in the environment of two-year-olds on the children's later aggression or altruism towards others. Once again, the immediate emotional reaction of children to others' anger was often distress. The distress elicited by exposure to anger tended to translate into more intense patterns of aggression towards peers in later play. Exposure to prosocial behavior between adults, on the other hand, resulted in a reduced frequency of aggressive initiations towards peers and a typically low-intensity pattern of aggression, but only when these events occurred prior to the anger simulation, not if prosocial interactions were introduced after the anger simulation. In contrast to their effects on aggression, others' angry or prosocial interactions had little direct impact on children's altruistic interactions with peers.

Children who participated in research in the home as toddlers were studied again at 6 to 7 years of age. Mothers again reported incidents in which children witnessed anger or affection between family members in the home. At this age, children were more likely to intervene empathically in others' angry disputes, but they were still clearly disturbed by these events. Patterns of responding to anger tended to be stable across the five-year span of the study. No developmental changes in responding to others' displays of affection were evident.

Significance to Biomedical Research:

The results of these studies suggest that temperamental dispositions contributing to behavior problems in young children may have their origins in children's experiential history as well as in biologically-based tendencies. Manipulations of the child's family environment through therapeutic interventions may thus have the potential to alter apparently innate temperament-related problems.

Proposed Course:

This is a final report.

Publications:

Cummings, E. M., Zahn-Waxler, C., and Radke-Yarrow, M. Young children's responses to expressions of anger and affection by others in the family. Child Dev. 52: 1274-1282, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02146-03 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Etiology of Problem Aggression in Early Childhood | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: E. Mark Cummings Other: Carolyn Zahn-Waxler Ronald Iannotti Marian R. Yarrow | Staff Fellow Research Psychologist Guest Worker Chief | LDP NIMH LDP NIMH LDP NIMH LDP NIMH |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: 1.56 | PROFESSIONAL: .81 | OTHER: .75 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The nature of <u>aggressive personality patterns</u> , the <u>stability of individual differences in aggression</u> , and factors contributing to the development and continuity of aggressive styles were studied in 1- to 2-year-old children in home and laboratory settings. The pattern of relationships between <u>aggression</u> , <u>altruism</u> , and <u>temperament</u> were different for boys and girls. A highly responsive temperament typified aggressive boys. They demonstrated reactive and intense styles of aggression, but they were also more altruistically responsive to others' distress, and more emotionally expressive. The behavior patterns of aggressive girls were not as distinct. Most notably, these girls frequently made reparations for their own acts of aggression than other girls. Even at this early age stable individual differences in aggression were apparent for both boys and girls, but evidence for stability was more consistent for boys. Aggressive children, particularly boys, differed the most from other children in aggression after exposure to emotionally threatening stimuli. Both aggressive boys and girls tended to have insecure <u>attachments</u> with the mother. A follow-up at four years of age is planned; biobehavioral aspects of aggression will also be examined at this time. | | |

Project Description:

Previous research suggests that aggressive behavior may have its roots in early childhood. The identification and delineation of early patterns of aggression could significantly advance both conceptual notions regarding the development of aggression, and clinical approaches to the treatment of problem aggression. The present research is concerned with: (a) the nature of early aggressive personality patterns, in particular, relationships between aggression, altruism, and temperament, (b) the stability of early individual differences in aggression, and (c) factors contributing to the development and continuity of aggressive styles.

Twenty-four 1-to 2-1/2 years old children were studied over a nine month period in the home, and an independent sample of 46 2-year-old children was seen in a series of laboratory sessions. Mothers trained as observers provided narrative reports of a variety of emotion-laden events, including aggression incidents, in the home. The laboratory sessions included the following: (a) a play session with a familiar male peer, (b) a play session with a familiar female peer, (c) a standard object conflict with an adult stranger, and (d) standard presentations of distress by the mother, adult strangers, and infants.

The pattern of relationships between aggression, altruism, and temperament were different for boys and girls. Aggressive boys were more likely than other boys to respond to another's resistance to aggression with heightened aggression, but they also more often responded altruistically (comforting, verbal sympathy, helping, sharing) to another's distress (pain, sorrow). Changes in the ambient emotional climate significantly influenced their aggression. In particular, exposure to threatening emotional events, such as adults arguing loudly or a brief separation from the mother in a strange situation, were followed by a marked increase in the intensity of their aggression. Aggressive boys were also more emotionally expressive (positive and negative affects) than other boys across a wide range of situations. In general, a highly responsive temperament typified aggressive boys; which was reflected by both a more reactive and intense style of aggression and a greater sensitivity to the emotional needs of others.

The behavior patterns of aggressive girls were not as distinct as in the case of aggressive boys. The only consistent trends were the following: Aggressive girls more frequently made reparations for their own acts of aggression than other girls. They were more likely to increase their aggression following exposure to brief separations from the mother in a strange situation.

Stable individual differences in aggression were apparent for both boys and girls, but the results were more consistent across independent situations for boys. Significant correlations were obtained for boys between the first and second 4 1/2 month period of home observations, and between each pair of independent laboratory sessions in which aggression was assessed. Significant correlations for girls were found between different home observation periods, but not between different laboratory sessions.

Analysis of the factors influencing the development and continuity of aggressive patterns has just begun, but some findings of interest have already emerged. Aggressive children, particularly boys, differed most significantly from other children in aggression after exposure to threatening emotional stimuli. However, as noted above, aggressive boys could also be highly altruistic in other emotional settings. Thus, aggressive boys would appear to have the potential to develop into highly sensitive and altruistic individuals, or antisocial individuals, depending upon the emotional environment in the home. Unfortunately, both aggressive boys and girls are already encountering more difficulty in their relationships with the mother, as indexed by relatively high incidences of insecure attachments.

Significance to Biomedical Research

The long-term goals of this project are an increased understanding of the contribution of biological and environmental factors to the early development of problem aggression, a biobehavioral index of risk for problem aggression which can be administered in early childhood and an early remediation program for children at risk for later problem aggression. The research to date suggests that the child's emotional relationship with the mother, the frequency of exposure to social stressors, and individual differences in autonomic reactivity each contribute to the early development of aggressive patterns.

Proposed Course

The first phase of the study, an examination of aggression in 1-to-2-year olds, is completed. The second phase of the study, a longitudinal followup when children attain 4-to-5-years of age, will begin later this year. It is anticipated that testing for this phase will take approximately one year, and data decoding, analysis, and the preparation of scientific reports will take approximately two years.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02147-03 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Effects and Determinants of Parental Methods for Controlling Children's Behavior | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Leon Kuczynski Visiting Fellow LDP NIMH | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .25 | PROFESSIONAL: .05 | OTHER: .20 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This study examines parental disciplinary techniques and other methods of influencing the behavior of children. The determinants of these techniques such as age of children, nature of children's transgressions and nature of parents' socialization goals as well as the effects of specific techniques on children's behavior are investigated. In the first phase of this study, 64 mothers and their 4-year old children were studied using a laboratory compliance task that assessed mothers' influence techniques and their effects on children's immediate and long-term compliance. Preliminary analyses indicate that parents use different patterns of techniques for long-term and short-term socialization goals. The second phase of the study will focus on parental use of discipline with children of different ages. Data will be obtained using naturalistic and laboratory methodologies. | | |

Project Description

This study investigates the determinants, content, and effects of parental disciplinary interventions with children. Parental control is an important focus of research on the environmental transmission of normal and dysfunctional behavior patterns. Many clinical interventions with problem families also directly focus on changing parental disciplinary strategies. However, limited conceptualizations and a lack of normative information about normal rearing processes--what techniques parents use, with what ages of children, for what purpose and with what effect--poses problems for effective clinical interventions and for diagnostic assessment.

One aim of the present study is to investigate parental control techniques in greater detail than previously and also as they vary as a function of children's age. A second aim is to investigate the determinants of parental choice of disciplinary strategy. Recent research suggests that a wide variety of techniques are part of normal parental repertoires but that parents discriminate among different situations in their use of these techniques.

In the first study, parental control strategies were investigated in a laboratory experiment with 64 normal mothers and their 4-year-old children. It was hypothesized that one determinant of parental choice of strategy is the long- or short-term nature of parental goals in particular situations. It was predicted that different patterns of techniques would be used with children depending on whether the goal was one of immediate or long-term compliance. Mothers' perceptions of the long- or short-term nature of the compliance that they were to elicit were experimentally determined. Preliminary analyses indicate that for both sexes, a pattern of strategies was used that included reasoning and character attributions for long-term compliance goals but a reliance on power-based techniques for short-term goals of immediate compliance. Mothers used more power based techniques with boys than with girls. Children's behavior reflected the appropriateness of mothers' choice of technique in the long-term condition where there was less negativistic behavior than in the short-term condition.

In the second phase of this project, data will be obtained from 3-, 7-, and 12-year-old children and their parents during naturally occurring disciplinary episodes and standard influence interventions in the home. Data on the determinants and effects of influence strategies will be obtained from detailed reports of mothers trained in observational recording (see Protocol 80-M-68 for details on method). Special attention will be paid to parents' spontaneous use of "reasoning" as well as to their reactions to selected chronic transgressions of their children.

Significance to Biomedical Research

The ultimate goal of this research is to understand the role of discipline in the development and treatment of childhood behavior disorders. By providing for adaptive and maladaptive patterns of discipline, this study will contribute to the development of prevention strategies, i.e., providing parents with

adaptive, effective patterns of discipline and the development of intervention strategies to assist parents in dealing with children with behavior problems.

Proposed Course

Data for the first phase of this project have been collected and are in the stage of analysis and preparation for publication. Planning for the second phase is now underway. The expected time for completion of the project will be 2 to 3 years.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02148 03 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Bilirubin and Affective Dysfunction in Pre-term Infants | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Carolyn Zahn-Waxler | Research Psychologist | LDP NIMH |
| Other: Sarah L. Friedman | Research Psychologist | NIE Wash. D.C. |
| COOPERATING UNITS (if any) National Institute of Education | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .05 | PROFESSIONAL: .05 | OTHER: .00 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Children born <u>premature</u> are <u>at risk</u> for problems in physical, cognitive and affective development. One potential biochemical precursor of <u>dysfunction</u> is (blood) <u>bilirubin</u> . Bilirubin, which causes jaundice, is neurotoxic: At high levels (>20 mg. %) it causes <u>mental retardation</u> . Bilirubin levels in preterm infants are often elevated. It is hypothesized that a significant portion of the diverse, adverse sequelae of preterm births may result from the presence of this neurotoxin, bilirubin, which <u>can result in brain damage</u> shortly after birth. The purpose of this research is to determine if the amount of impairment in early affect expression (<u>cries</u>), <u>neurological performance</u> and <u>visual function in preterm infants</u> is associated with concentration of <u>bilirubin in the blood</u> . | | |

Project Description:

Preterm infants constitute a population that is at risk in several respects. They experience significant (a) mortality and morbidity, (b) physical health problems, (c) impaired inferential skills, (d) visual problems, (e) difficulties in regulation of affect. While some of the correlates of preterm births have been identified and while many of the problems associated with early birth are known, direct causal connections between specific pre- and post-natal processes and specific biological and behavioral outcomes are difficult to infer. The neurotoxin bilirubin, which damages many regions of the brain shortly after birth might be implicated in a significant portion of the adverse sequelae of preterm births. The purpose of this research is to determine if the amount of impairment in affect expression, motor performance, and sensory performance of preterm infants can be predicted from the concentration of bilirubin in the blood.

Behavioral data were obtained on 45 preterm infants in individual testing sessions. Each child was tested at expected date of birth. The measures included visual attention, a repetitive visual stimulus (1) orientation, attention level and response decrement), (2) neurological function (the Parmelee neurological examination), and (3) affect expression, measured in terms of (a) irritability and soothability, and (b) infant cries tape-recorded during the neurological examination. The biochemical and medical data were obtained from hospital records.

The study of preterm infant cries may begin to provide a link between some of the biological and behavioral problems faced by significant numbers of preterm infants. The preterm cry has been described as aversive, stressful, and disorganized. Some studies find preterm infants to be at risk for abuse and neglect: Their disorganized affect might elicit primitive, disorganized and aggressive behaviors in some caretakers. For other caretakers, the cry may signal that special care and support are needed. Our understanding of these interactive processes should be enhanced by increased knowledge of the origins of the affective expressions that the preterm child brings to its environment.

Significance to Biomedical Research

This research investigates the extent to which emotional and neurological problems experienced by preterm children may result from brain damage caused by postnatal biological disturbances. If high levels of the neurotoxin, bilirubin are closely linked to early developmental problems, there are implications for choice of therapeutic interventions (e.g., the degree to which phototherapy for jaundice is selected as a treatment procedure).

Proposed Course

A specialist in spectrographic and auditory analyses of cry sounds has just completed coding the cries, assessing degree of abnormality of preterm cry sounds. The data are currently being analyzed and the project will be completed in 1 to 2 years.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02149-03 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Children's Reactions to Infant Cries | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Carolyn Zahn-Waxler OTHER: Sarah L. Friedman E. Mark Cummings | Research Psychologist Guest Worker Staff Fellow | LDP NIMH LDP NIMH LDP NIMH |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .05 | PROFESSIONAL: .05 | OTHER: .00 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Infant cries</u> are complex stimuli, capable of eliciting both <u>altruistic and aggressive behaviors</u> in others. Cries of preterm infants have been described as aversive, and as potential <u>elicitors of child abuse</u> . Children's response patterns to preterm and full-term infant cries were examined. Their behavior on hearing a cry in the next room, their response to mother and child in their presence, ratings of their own feelings upon hearing the cries, and their verbalizations about the cries were obtained. Children's self-reports of feelings of empathy, verbalized intentions to help, observed affective arousal and actual helping responses were common responses to cries at all ages. In addition, there were significant age increases in caregiving interventions toward crying infants. Children showed similar feelings of empathy and similar rates of prosocial interventions in response to <u>preterm and full-term infant cries</u> . Yet, most children readily distinguished between the two cry types along dimensions of abnormality and illness. | | |

Project Description:

Within an ethological framework, infant crying is viewed as a distress signal which serves as a direct releaser of adaptive, caregiver behaviors in both humans and animals. Crying thus protects the individual and helps to ensure survival of the species. The cry can also be conceptualized as an activator of positive and negative emotions in other persons which, in turn, influence their empathic caregiving or rejecting behaviors toward infant. The cries of premature infants sometimes have been characterized as aversive stimuli that have an increased likelihood of eliciting aggressive, abusive behavior from caregivers. This research examines children's responses to the cries of preterm and full term infants.

Sixty middle-class boys and girls were studied at three ages: 4 to 6, 6 to 8, and 10 to 12 years. Each child was seen in the laboratory. During the sessions a staged-incident occurred: A (tape-recorded) cry was heard in an adjacent room. Then a mother, carrying her infant, entered to look for her "crying" baby's bottle. For half of the children the cry was that of a premature infant; for half the children the cry was that of a full-term infant. The child's responses were recorded. Later in the session each child listened to a tape-recording of a premature and a full-term infant cry, and rated his/her own feelings (fear, anger, empathy) when listening to the cries. The cries were then replayed and children were asked to describe them.

Children's self-reports of feelings of empathy, observed tension/anxiety, verbalized intentions to help and actual helping responses were common responses to cries at all ages. In addition, there were significant age increases in caregiving interventions toward crying infants. Observed tension/anxiety in response to overhearing an infant cry was negatively related to (i.e., interfered with) subsequent direct helping efforts. Empathy and helping did not occur differentially in response to preterm or full-term cries. Young children expressed more anger in response to full-term than to preterm cries. This is congruent with our earlier reported study of parents' responses to cries in which preterm cries were not consistently perceived more negatively than full-term cries by mothers. In general, children's predominant behaviors toward crying infants were altruistic while their emotions showed a mix of empathic arousal and anxiety. Most children described the preterm and full-term cries in different terms; preterm cries were frequently described along dimensions of abnormality, illness and stress. Often, children's statements also reflected awareness that different cries can elicit different feelings and behaviors in others.

Significance to Biomedical Research:

The ways to which infants at risk (e.g., preterm infants) are perceived and understood affects the ways in which these children are treated, not only in medical settings but in their homes as well. This research identifies the abilities of children (a) to detect signs of abnormality, illness, and stress in preterm infants and (b) to provide care for infants perceived as ill. The findings have implications for ways in which siblings of at-risk infants

(significant persons in the infant's environment) can be taught to interact with ill infants in order to promote adaptive emotional growth.

Proposed Course:

A manuscript has been submitted for publication. This is a final report.

Publications:

Zahn-Waxler, C., Cummings, E. M., Welsh, J., and Friedman, S. Children's responses to cries of premature and full term infants. In Kirkland, J. (Ed.), Cry Research. Palmerston North, New Zealand: Massey University Press, 1981, 3, 2.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02150-03 LDP |
| PERIOD COVERED October 1, 1980 through September 30, 1981 | | |
| TITLE OF PROJECT (80 characters or less) Adjustment to Stress in Early Adolescence: Environmental and Organismic Factors | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Editha D. Nottelmann | Staff Fellow | LDP NIMH |
| OTHER: C. Jean Welsh | Psychologist | LDP NIMH |
| COOPERATING UNITS (if any) Montgomery County Public Schools | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: 2.40 | PROFESSIONAL: .90 | OTHER: 1.50 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This study is focused on the relationship between children's <u>adjustment</u> in and outside of school and <u>discontinuity</u> in their lives. Children are studied in <u>transition</u> from elementary school to middle or junior high school and from <u>childhood</u> to <u>early adolescence</u> . Children's adjustment in <u>school</u> before transition and their ability to withstand the <u>stress</u> of the many changes they encounter during transition are examined in children's self-reports and reports from their teachers and peers. Measures include (a) <u>children's perceptions</u> and <u>teachers' ratings</u> of their cognitive, social, physical and general competence, (b) children's <u>self-image</u> , and (c) children's peer relations in school. They are examined in relation to (a) indices of children's <u>physical maturity</u> , (b) teachers' ratings of children's academic standing, and (c) children's perceptions of their school environment. For a group of children, adjustment and ability to withstand the stress of these changes are examined also as a function of their lives outside of school, primarily their <u>family</u> and <u>peer relationships</u> . | | |

Project Description:

This is a large-scale longitudinal study that is focused on the relationship between children's adjustment in and outside of school and experience with discontinuity in their lives. Children are studied in transition from elementary school to middle or junior high school and from childhood to early adolescence. Their adjustment and ability to withstand stress are examined across school settings, from their own perspective as well as from that of their teachers and peers; they are examined also as a function of their family and peer relationships.

The transition from primary to intermediate school has been selected for study because it represents significant imposed change for a large number of children in our society and because it coincides with the onset of puberty for many of these children. It involves changes that are sufficiently far reaching to constitute a sharp discontinuity and a source of stress in children's lives. Many developmental and behavioral problems of children appear at this time, when the demands of new environments and the developmental demands and issues of later childhood and adolescence intersect.

We are concerned with how children feel about themselves during this period of transition, with their ability to make friends and find peer support, and their general psychological well being. Also, we are concerned with how their adjustment is influenced by major change in their status, peer networks, and environmental demands. Measurements are made prior to transition, early in the period of transition, and again later, after they have had an opportunity to adapt to their new environment.

For approximately 430 children, the relationship between adjustment and discontinuity is examined in the context of their peer-school environment. A subgroup of 160 children also participated in in-depth interviews about their peer-family environments outside of school, and their parents completed questionnaires to provide complementary information.

Processing of the data collected in schools at three times of measurements across a one-year period has been completed; processing of data collected subsequently by interview and questionnaires continues. Statistical evaluation of the longitudinal data collected in the schools and preparation of research reports for publication is under way.

Early analyses indicate stability across time and school environments in children's self-perceptions in general, but relatively low self-assessment among a subsample of children who are "off time" in physical maturation in relation to their peer group (i.e., identified as > 1 standard deviation away from their group mean in height/weight ratio); in particular, among "more mature" girls and "less mature" boys. School transition had no impact on these children's perceptions of their academic competence -- both children who did and children who did not change schools rated themselves lower than their peers. Transition had an impact on their perceptions of their social and physical competence; that is, those "more mature" girls and "less mature" boys who changed schools rated themselves lower than those who did not change

schools. Teachers' ratings indicate that they did not see these children as different from their peers except in physical competence (sports). However, "off-time" children generally reported relatively low self-esteem.

Early analyses also indicate that "short-for-age" children are vulnerable. In a comparison of short and tall stature children (i.e., children identified as one standard deviation away from their group mean), short stature children reported lower self-esteem and academic competence than tall stature children. The children's assessments of their academic competence were supported by their teachers, who gave short stature children much lower ratings than tall stature children.

The data collected in the school and the additional interview data collected in the home represent a rich source of information about the every-day lives of children entering early adolescence. Even though transition from childhood to adolescence and early adolescence in itself are recognized as sensitive periods of development, we know very little about them. Research on adolescence in the past has concentrated on late adolescence.

Significance to Biomedical Research:

Rapid biological changes and cultural pressures are sources of stress and anxiety that appear to be implicated in the high incidence of affective and behavioral disorders during adolescence. This project will provide a much needed normative data base on early adolescent development for endocrinologists concerned with problems related to "off time" adolescent growth and pubertal development; and also for professionals concerned with mental health assessment and prevention of disorders in adolescents.

Proposed Course:

Data analyses will continue. The dissemination and publication of the findings has begun.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02152-03 LDP | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Discipline and Parental Control in Normal Families and Families with Affective Disorders | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Leon Kuczynski</td> <td style="width: 25%;">Visiting Fellow</td> <td style="width: 20%;">LDP NIMH</td> </tr> <tr> <td>Other:</td> <td>Marian Radke-Yarrow</td> <td>Chief</td> <td>LDP NIMH</td> </tr> <tr> <td></td> <td>Carolyn Zahn-Waxler</td> <td>Research Psychologist</td> <td>LDP NIMH</td> </tr> </table> | | | PI: | Leon Kuczynski | Visiting Fellow | LDP NIMH | Other: | Marian Radke-Yarrow | Chief | LDP NIMH | | Carolyn Zahn-Waxler | Research Psychologist | LDP NIMH |
| PI: | Leon Kuczynski | Visiting Fellow | LDP NIMH | | | | | | | | | | | |
| Other: | Marian Radke-Yarrow | Chief | LDP NIMH | | | | | | | | | | | |
| | Carolyn Zahn-Waxler | Research Psychologist | LDP NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology SECTION | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL Staff Years:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td>.55</td> <td>.35</td> <td>.20</td> </tr> </table> | | | TOTAL Staff Years: | PROFESSIONAL: | OTHER: | .55 | .35 | .20 | | | | | | |
| TOTAL Staff Years: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | |
| .55 | .35 | .20 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> This investigation is concerned with the measurement and conceptualization of parental influence in families with normal and depressed mothers. This study is part of a series of investigations, the basic research paradigm for which is described in Annual Report MH 02144. Children's <u>misbehaviors</u> and mothers' discipline and <u>influence techniques</u> are observed during a series of half-day sessions in a laboratory setting that simulates a home environment. Data on maternal influence will be based on naturally occurring controls placed on children in the laboratory setting as well as experimentally elicited influence interactions. The range of control techniques used by mothers, the effects of these techniques on children's behavior and the role of children in influencing the kinds of discipline used by parents are investigated. Another focus is on the relationship between specific kinds of misbehaviors and the kinds of controls elicited by them. The acquisition of specific aspects of self-control are examined longitudinally in order to document long-term processes in the development of selected behaviors. </p> | | | | | | | | | | | | | | |

Project Description:

This study is concerned with the conceptualization and measurement of parental discipline and control of children's behavior in families with normal and depressed mothers. This study is part of a series of investigations, the basic paradigms for which is described in Annual Report MH 02144. In previous research, discipline has been globally assessed and the content of parental interventions--how and when do normal and depressed parents intervene in children's behavior--is not known. Both the content of parental intervention techniques and the content of the behaviors that they attempt to control are examined. Previous research has relied on a unidirectional model in which the child's role in the interaction is ignored. In the present study parental control will be examined in relation to children's behaviors to provide a basis for understanding the frequency of parental interventions and to examine the appropriateness of their methods. By studying whether specific categories of children's behaviors elicit characteristic patterns of discipline, it may be possible to move away from descriptions of parents in terms of what techniques they use to a consideration of how they use their techniques and whether specific techniques are used appropriately.

Most of the available data on the effects of discipline are based on discrete sequences of disciplinary interactions. Usually, the effects of single administrations of control techniques on children's immediate compliance are studied. In the natural environment, however, children are likely to commit the same transgressions repeatedly over time, on each occasion eliciting different controls from their parents before the behavior finally comes under control. In this study, children's acquisition of selected aspects of self-control and mothers' reactions to recurrences of the same misbehaviors will be observed over a period of several days. This longitudinal design will provide a preliminary model for long-term processes in the acquisition of behavior and for understanding the effects of repeated disciplinary interventions.

Significance to Biomedical Research:

Children of depressed parents have been found to be at greater risk for psychopathology and behavioral disorders than children of normal parents. Early research suggests that aberrant disciplinary practices comprise one of a pattern of environmental factors to which such children are exposed. The present study investigates the role of discipline in families with affective disorders both as a process that may predispose children to behavioral problems and also as a focus for new forms of prevention and treatment.

Proposed Course:

Data for this project and for other projects in this series are being collected from observations of normal and clinically depressed mothers and their children in a series of half-day sessions in a laboratory setting designed to simulate a home environment. Data collection and analysis is underway. Instruments for coding the videotaped data are being developed.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02153-03 LDP | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Maternal Recall of Child's Early Experience | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Penelope K. Trickett</td> <td style="width: 40%;">Senior Staff Fellow</td> <td style="width: 10%;">LDP NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Marian R. Yarrow</td> <td>Chief</td> <td>LDP NIMH</td> </tr> <tr> <td></td> <td>Carolyn Z. Waxler</td> <td>Research Psychologist</td> <td>LDP NIMH</td> </tr> </table> | | | PI: | Penelope K. Trickett | Senior Staff Fellow | LDP NIMH | OTHER: | Marian R. Yarrow | Chief | LDP NIMH | | Carolyn Z. Waxler | Research Psychologist | LDP NIMH |
| PI: | Penelope K. Trickett | Senior Staff Fellow | LDP NIMH | | | | | | | | | | | |
| OTHER: | Marian R. Yarrow | Chief | LDP NIMH | | | | | | | | | | | |
| | Carolyn Z. Waxler | Research Psychologist | LDP NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology SECTION | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL Staff Years:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 34%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">.22</td> <td style="text-align: center;">.12</td> <td style="text-align: center;">.10</td> </tr> </table> | | | TOTAL Staff Years: | PROFESSIONAL: | OTHER: | .22 | .12 | .10 | | | | | | |
| TOTAL Staff Years: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | |
| .22 | .12 | .10 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> In this study, the relation between <u>mother's recollections of certain child behaviors and child-rearing techniques</u> and parallel information obtained at an earlier time when the behaviors were current is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology. Although previous research has demonstrated that in general the correspondence between contemporaneous and retrospective data is low, there is still much to be learned about what determines when mother's recollections are more or less accurate. (1) Are certain kinds of information recollected more accurately than others? (2) How does the mother's current view of the child color her memories? The subjects are mothers of 26-month-old children who are part of a study of etiology of behavior problems. The data sets that are compared are: (a) mother's observations and investigator ratings when the child was 18 months of age, and (b) mother's retrospective reports given 6 to 8 months later about the 18-month period. </p> | | | | | | | | | | | | | | |

Project Description:

Much of the knowledge in the field of child development on childrearing techniques has been derived from information obtained by interviewing mothers. Many of these interviews have been retrospective, asking the mother to recall certain characteristics of her child or her child-rearing techniques when her child was at a younger age. Other interviews concentrate on the present but still rely on retrospection to the extent that they require the mother to use memories of previous experiences in order to make generalizations about her own or her child's typical behavior. In this study, the relation between mother's reconstructions of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology.

Previous research (Yarrow, Campbell, & Burton, 1970) has demonstrated that the correspondence between mothers' recollections and parallel information obtained at an earlier time is often low and that systematic biases in retrospection can occur. However, there is still much to be learned about the factors that determine when mothers' recollections and generalizations are apt to be more or less accurate. For example, are certain kinds of information recollected more accurately than others? How does the mother's current relationship to the child color her memories?

Mothers in the current research are part of a clinical study of etiological factors in the development of behavior problems of children. The children are now 26 to 28 months of age. The baseline data are (a) observational records by mothers who have been trained as observers and have been providing, on a longitudinal basis (10 to 24 months), rich data on their children's responses to emotions, transgressions, and prosocial behavior and on their own discipline techniques, (Z01 MH 00275), and (b) investigators' assessments of the mother-child relationship and of child behavior. When each child was 26 months of age, the mother reconstructed her child's and her own behavior when the child was 18 months old. She also filled out a standard child-rearing attitudes measure and a mood checklist. The investigator made ratings of current mother-child relationships at that time as well. The several sets of data will be compared for correspondences and differences. The mediating effect of parental mood and child rearing attitudes on accuracy of retrospection will also be examined.

Significance to Biomedical Research

This study will result in a delineation of both the types of information that are more or less accurately remembered by mothers and the characteristic of the mother's personality, attitudes and values which affect retrospection. Such a delineation will aid the development of subsequent biomedical and psychiatric research which relies on maternal retrospection as the method of choice for obtaining necessary information about children's past histories.

Proposed Course

Data collection has been completed. The coding and analysis of the data are underway. The project should be completed within one or two years.

Publications:

None

Project Description:

A fundamental question in children's services is how to distinguish behavior problems that are transitory from those that are more persistent. Efforts addressing this issue have provided conflicting results. Some studies report that behavior problems of children change over time, while others report that behavior problems show little change in follow-up analyses.

In addition, previous analyses of data on childhood disorders have mainly focused on global measures of behavior. The present study was designed to make a molecular analysis of individual behavior problems using a standardized instrument for assessing child behavior problems and competencies (CBCL). The data were obtained, in previous studies, on several hundred children in an outpatient clinic, at intake and after treatment.

The design entails an analysis of change or stability in individual behavior items. The instrument used is a standardized inventory of adaptive and maladaptive child behavior consisting of 118 behavior items designed to be rated by parents on a 3-point scale. Two age groups, 6-11 and 12-16, of each sex were examined. The scale was completed by the parents of 468 children at the time of the children's intake and again 6 months and 18 months thereafter. Statistical analyses have been performed to determine which problems are transitory and which are resistant to change.

Our findings show that the question of stability and change of clinically relevant behavior cannot be answered in an all-or-none fashion. Some deviant behaviors showed great stability with respect to both rank ordering and absolute level. Other behaviors showed major changes in level, but the changes in level were so uniform within a cohort that individual children retained stable rank orders over time. Still other behaviors remained at a constant level within a cohort, but individual children changed their rank ordering relative to peers. Some behaviors showed considerable change, both in terms of children's rank order and the level prevailing within a cohort.

Two items: 55. Overweight; and 81. Steals at home, were stable in terms of correlations $\geq .50$ for both sexes aged 6 to 11 and 12 to 16. Also, there were behavior problems with a stable rank order that were the same for both sexes but only at certain ages. Six- to eleven-year-old boys and girls had five behavior problems with stable rank order and nonsignificant change in mean scores: 44. Bites fingernails; 55. Overweight; 81. Steals at home; 90. Swearing; and 98. Thumb-sucking. Twelve- to sixteen year olds of both sexes had three behavior problems with a stable rank order and nonsignificant change in mean scores: 55. Overweight; 56e. Skin problems; and 81. Steals at home.

Six- to eleven-year-old girls had more stable internalizing behavior problems than did boys, and both sexes had about the same number of stable externalizing behavior problems. Boys, however, had more stable externalizing than internalizing behavior problems, while girls had about the same number of stable externalizing and internalizing behavior problems with correlations $\geq .50$.

Twelve- to sixteen-year-old girls had more externalizing behavior problems than did boys, but boys and girls had an almost equal number of internalizing behavior problems with correlations $>.50$ at both follow-ups. Girls in this age group also had more externalizing than internalizing behavior problems, while boys had about the same number of externalizing and internalizing behavior problems with correlations $>.50$ at both follow-ups with nonsignificant change in mean scores.

There were no behavior problems with low stability coefficients that were the same for both boys and girls, either in the younger or older group. Also, all the behavior problems with low stability coefficients at both follow-ups showed no significant changes in mean scores with the exception of 101. Truancy; and 105. Alcohol or drugs. These items represented very unstable behavior problems for girls aged 6 to 11. While these items did not maintain stable rank order, average scores for the entire cohort did change significantly. Thus, younger boys, older boys and older girls had behavior problems with low stability coefficients at both follow-ups and similar mean scores at both follow-ups.

Significance to Biomedical Research:

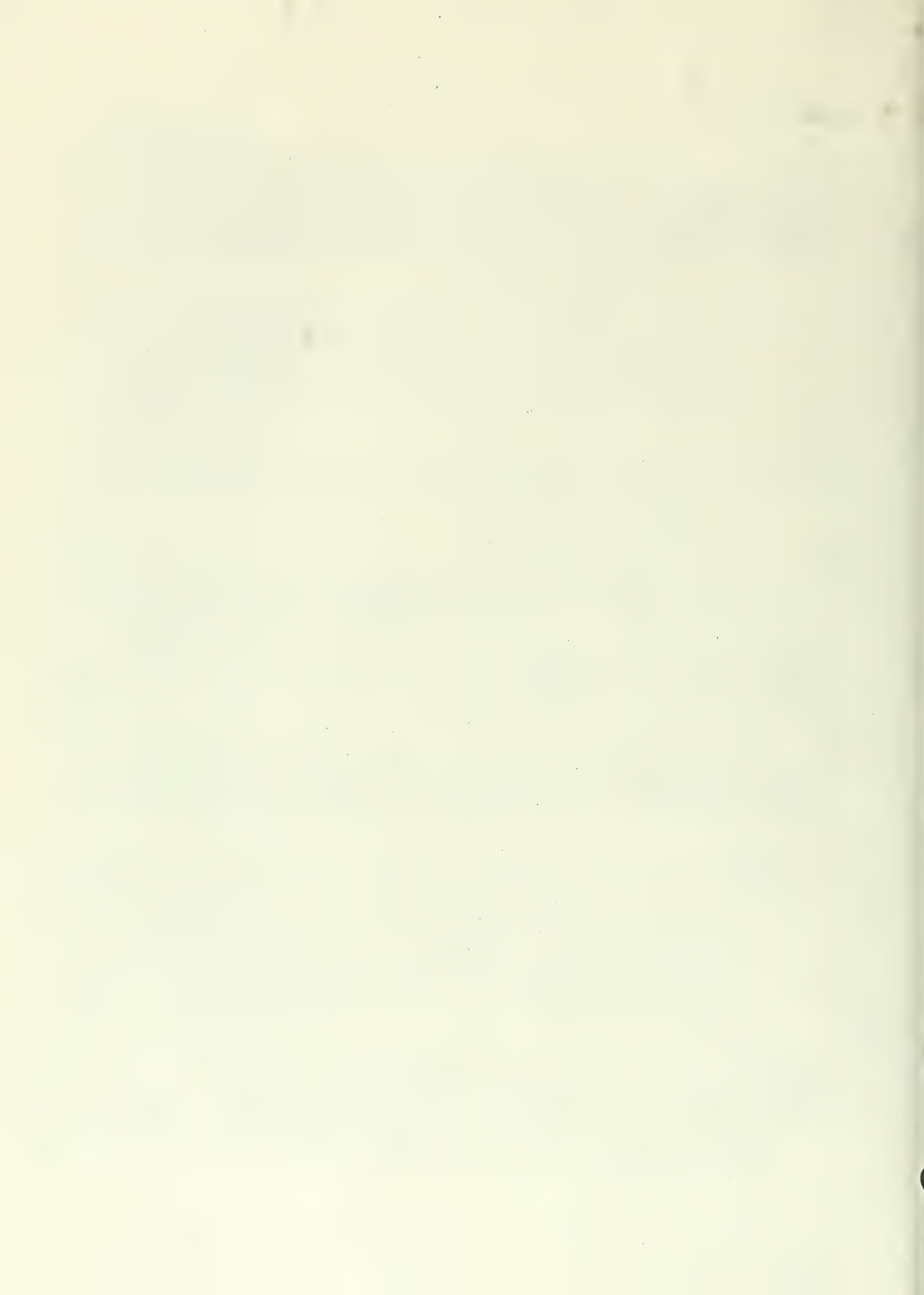
This study is the first to examine childhood disorders by assessing stability and change of a large number of behavior problems of children referred for mental health services in terms of individual behavior problems. It offers an experimental approach for the assessment of the therapeutic outcome of clinically referred children by detecting the most critical behavior disorders of children.

Proposed Course:

It is anticipated that analyses will be completed within the year and a manuscript based on this publication will be submitted for publication -- Stability and Change in Behavior Problems of Clinically Referred Children.

Publications:

None



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02155-03 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Perceptions of Affect in Normal and Depressed Families | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Carolyn Zahn-Waxler Other: Marian Radke-Yarrow Leon Kuczynski Leon Cytryn Donald McKnew Linda Stern Mark Cummings Ronald Iannotti | Research Psychologist Chief Visiting Fellow Research Psychiatrist Research Psychiatrist Student Scientist Research Psychiatrist Guest Worker | LDP LDP LDP BPB-LDP LDP LDP LDP |
| NIMH NIMH NIMH NIMH NIMH NIMH NIMH | | |
| COOPERATING UNITS (if any) Biological Psychiatry Branch | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: 1.78 | PROFESSIONAL: .28 | OTHER: 1.50 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) How do the mood disorders of parents influence how they and their children process and react to the emotions of others? This is a study of <u>perceptions of others' emotions</u> by parents and children in normal and depressed families. Structured tests (photographs of infants and picture stories about distress) are used to assess mothers' perceptions of infants' emotions and children's interpretations of affect and social interactions of others. Preliminary analyses indicate differences in children with parents from different diagnostic categories. <u>Children of manic-depressive parents</u> are overly emotionally reactive to the distress states of others, with particular sensitivity to states of anger and aggression. Children with depressed mothers are less able to communicate about and interpret sadness. | | |

Project Description

How do the mood disorders of parents influence how these children process and react to the emotions of others? The first project (in collaboration with the University of Colorado Medical Center) consists of systematic comparisons of depressed (unipolar and bipolar) and normal mothers' perceptions and interpretations of affect expression in very young children. Disturbances in the affective experiences of depressed parents could result in their having distorted perceptions of others' emotional states and expressions (e.g., perceiving unusually high or low levels of sadness, pleasure, or anger in young children). This could, in turn, influence parent-child interaction. The second project examines perceptions of affect in early school-age children from depressed and normal families.

In Study 1, the Denver Free Response Labeling Pack is administered to mothers. The stimulus materials consist of a set of 35 photographs of 12 month old infants showing a range of affective expressions. Some photographs contain neutral facial expressions and hence permit assessments of the projection of different emotions. Emotions commonly identified in the infant photographs include (1) interest, (2) enjoyment/joy, (3) distress/anguish, (4) fear/terror, (5) anger, and (6) surprise. Depressed and normal mothers will be compared on the frequencies and kinds of emotions they attribute to very young children. The diagnostic screening procedures, sampling, and observational techniques are described in Project Report Z01 MH 02146.

In Study 2, the perceptions of affect of the early school age children (5-8 years) of normal and depressed mothers are compared. In individual testing sessions children are told a series of distress stories (a mother is angry with her family and departs; two children fight and one is injured; a parent receives bad news, etc.). Children's perceptions and emotional understanding of these stories are probed. The affective content, themes, and coping strategies of children (e.g., anger, guilt, sadness, happiness, denial, hypersensitivity) are scored by two independent observers, blind to the diagnostic category of the parents. Preliminary analyses indicate significant differences between children with parents in different diagnostic categories: Children from bipolar (manic-depressive) families were the most likely to show hypersensitivity to distress stories and they were the most likely to identify themes of punishment and aggression in the distress stories. Children with depressed mothers scored very high on denial, i.e., they had considerable difficulty talking about emotions and emotional situations.

Significance to Biomedical Research:

As part of the research program on depressed mothers' rearing behaviors, the development of techniques is needed for assessing affective communication between parent and child. The adaptation of existing methods and the development of new procedures has been accomplished. (See MH 02156 for further substantive information.)

Proposed Course:

This is a longitudinal study in which families are followed over a period of 3 years. The sample base which is now about 40 will be doubled. It is anticipated that 4 to 5 years will be required for completion of data collection.

Publications:

NONE

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|--|---|---|-----|---------------------|-------|-----|------|--|---------------------|-----------------------|-----|------|--|----------------|-----------------|-----|------|--|-------------|-----------------------|-----|------|--|---------------|-----------------------|-----|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02156-03 LDP | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Studies of Child Rearing: Rearing by Normal and Depressed Mothers and the Emotional-Social Development of Their Children | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Marian Radke-Yarrow</td> <td style="width: 30%;">Chief</td> <td style="width: 10%;">LDP</td> <td style="width: 10%;">NIMH</td> </tr> <tr> <td></td> <td>Carolyn Zahn-Waxler</td> <td>Research Psychologist</td> <td>LDP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Leon Kuczynski</td> <td>Visiting Fellow</td> <td>LDP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Leon Cytryn</td> <td>Research Psychiatrist</td> <td>LDP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Donald McKnew</td> <td>Research Psychiatrist</td> <td>LDP</td> <td>NIMH</td> </tr> </table> | | | PI: | Marian Radke-Yarrow | Chief | LDP | NIMH | | Carolyn Zahn-Waxler | Research Psychologist | LDP | NIMH | | Leon Kuczynski | Visiting Fellow | LDP | NIMH | | Leon Cytryn | Research Psychiatrist | LDP | NIMH | | Donald McKnew | Research Psychiatrist | LDP | NIMH |
| PI: | Marian Radke-Yarrow | Chief | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Carolyn Zahn-Waxler | Research Psychologist | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Leon Kuczynski | Visiting Fellow | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Leon Cytryn | Research Psychiatrist | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | Donald McKnew | Research Psychiatrist | LDP | NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL Staff Years: 3.10 | PROFESSIONAL: .60 | OTHER: 2.50 | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The rearing behavior of mothers diagnosed (SADS) as depressed and mothers without RDC diagnoses and the behavior of their young children is investigated. Mothers and their 2- and 5-year-olds are observed for 3 half-days in a home-like laboratory apartment in which naturally occurring rearing demands and interactions are experienced, and into which experimental conditions are also introduced. Behavior is measured in terms of cognitive content, affect, communication, and interaction. <u>Direct observations</u> are supplemented by interview data. Psychiatric assessments are made of each child, independent of the observational data. Followup measurements are made over a 2 to 3 year period. The <u>transmission of patterns of behavior</u> and the interaction of biological and environmental variables are the focus of the study. Biological assessment procedures will be introduced at the time of follow-up. | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Children of parents with affective disorders are at greater risk for the development of psychopathology than children of parents who have no psychiatric diagnosis. Both genetic and environmental explanations have been proposed. Although there is agreement on the interactive effects of genetic and environmental influences, few studies have investigated their interaction. In particular, little systematic research has focused on how parental depression impinges on children in the rearing process. Moreover, measures and conceptualizations of the behavioral environment have been inadequate.

The research purpose is to investigate the environmental transmission of behavior patterns in families with and without parental psychopathology. Parents diagnosed as depressed (bipolar, major unipolar, minor unipolar and intermittent) and normal parents and their 2-year-old and 5-year-old children are the research participants. Parents are diagnosed using Spitzer and Endicott's (1978) Schedule for Affective Disorders and Schizophrenia, life-time version (SADS-L) and the associated Research Diagnostic Criteria (RDC). In the present study, the mother's rearing --her actions and her interactions with her children--and the children's functioning are examined directly.

Although it is hypothesized that depressed and normal mothers will differ, as groups, on dimensions associated with depression (apathy, brooding, self-preoccupation, etc.), it is also expected that these dimensions will not cleanly differentiate the two groups, and will not adequately characterize the rearing environment either in terms of mother-child interactions or in terms of the kinds of behavior settings provided by the mothers. Mothers' behavioral qualities are, therefore, considered in terms of direct and indirect influences on the child. Indirect influences are those aspects of stimulation (e.g., mother's energy level, content of mother's activities, affective expression) that are not specifically directed to the child but have the potential to impact on child development. An important category of indirect maternal influence considered in this study is the mother's ongoing affective expression. Direct influences are the behaviors specifically directed to the child in verbal or overt behavioral manifestations.

It is expected that affect-linked differences in both normal and depressed rearing conditions will have significant effects on children's learning about the self, on the form and patterning of their interpersonal behaviors, and on their coping with environmental demands. One interest is in examining children's means of coping with parent's depression. Another interest is in the developmental course of children's behavior in relation to maternal rearing. Ages 2 to 3 and 5 to 6 were chosen as the periods in development at which significant changes are normally occurring--in cognition, verbal facility, dependence on parent, exposure to a non-family world. The design of the present study allows one to observe rearing patterns of the mother and transitions and transformations in the developmental process across this span of years.

Families are visited in the home to provide them with orientation regarding the study, obtain specified information on the family, and instruct mothers in the use of self-report forms that provide information on (a) social contacts of previous day; (b) crisis events of previous day, and (c) current moods. These forms are filled out at the time of each research session at NIMH.

Mothers and children are observed with each other, in a laboratory, an apartment that is an informal, home-like setting. Usual daily routines, demands, and interactions, take place (eating, playing, resting, toileting, telephoning, watching TV, etc.). Each day has, however, by experimental design, certain standard events (e.g., stimulus events occur such as mother's brief departure, mother's inaccessibility, opportunity for enjoying a shared activity, required limit-setting, handling frustrations, etc.). On the first two half days mother and 2-year-old come to the laboratory. On the third day, the mother, her 2-year-old and an older sibling (5 to 8 years) come to the apartment.

On the fourth day, each child is seen by a psychiatrist (a planned play session for the younger child and an interview (the CAS) for the older child). The psychiatrists are blind to the diagnostic category of the mother and the status of the sibling. The older child is also given projective tests to measure guilt, empathy, aggression, and expectations of harm or good. The mother is interviewed and also fills out the Achenbach Behavior Check List on each child.

At the end of the fourth session a staff round table is convened to formulate a clinical report on the children and mother, to determine the feedback that will be given to the mother, and to decide on necessary referrals or interventions in cases warranting some action.

Each family will be seen at intervals over a period of two to three years. On the return visits parts of the initial assessments are repeated: rearing-setting modified for developmental level, psychiatric assessments, mother's self-assessments, Achenbach Check List reports. Biological assessments are projected in the followup procedures. These assessments will be done in collaboration with other laboratories. To date approximately 40 families have been seen through the first phase of the study. Observations based on these very preliminary data are the following: 87% of the 5- to 8-year-old children of the lower economic class depressed mothers, 40% of the children of the middle class depressed mothers, and 33% of the children of the middle class normal mothers received diagnoses. In the lower economic group, diagnoses were of a depressive disorder in 71% of the cases; in the middle-class group diagnoses were of a depressive disorder in 29% of the cases. Depressive items on the Achenbach Scale for the children of the middle class depressives reflected self-criticism, guilt, and concerns with perfection. For the children of the lower class, the items revealed themes of ambivalence, dependency, and despair. There is a significant relation between aggression and depression in the children of the depressed mothers. These findings must be regarded as tentative. It is still too early in the research to undertake major analyses of the data.

Significance to Biomedical Research:

The rearing study should provide us information concerning (1) the prevalence of childhood disorders in families with parents who have a variety of affective disorders; (2) the development of early childhood disorders and their course over time; (3) specific environmental factors in these families that seem to predispose children to psychological illness or to protect them from it; and (4) the factors in the children that both make them vulnerable and protect them from such environments. This is one of the first attempts to investigate in a direct way the functioning of depressed adults in their parent roles and the effects on their offspring, from the earliest ages. Initial results indicate findings that have relevance for prevention and treatment: (1) prevention or amelioration of children's problems through early detection; and (2) better informed family treatment and counseling and child therapy.

Proposed Course:

This is a longitudinal study in which families are followed over a period of 3 years. The sample base which is now about 40 will be doubled. It is anticipated that 4 to 5 years will be required for completion of data collection.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02157-03 |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Developmental Evaluation of Infants on Chloride Deficient Diet | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Howard A. Moss | Guest Worker | LDP NIMH |
| OTHER: Van S. Hubbard | Clinical Associate | PMB NIAMDD |
| COOPERATING UNITS (if any) Pediatric Metabolism Branch, NIAMDD | | |
| LAB/BRANCH Laboratory of Developmental Psychology SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .30 | PROFESSIONAL: .10 | OTHER: .20 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) In 1979 it was reported that a group of infants who were receiving a <u>Soy formula</u> were not behaving and developing according to normative expectations and were exhibiting a "failure to thrive" type syndrome. Analysis of this infant formula revealed that it was deficient in chloride and that many of the infants fed this formula were suffering from metabolic alkalosis and chloride and potassium deficiencies. Changes to a properly balanced formula improved their condition. There is some question, however, whether or not any permanent damage was sustained. A study of these infants investigates the possibility of <u>long-term effects</u> from this <u>dietary deficiency</u> . This research focuses on the child's intellect, behavioral development, and on the parents' child rearing practices. | | |

Project Description:

In 1979 it was reported in an investigation conducted by the Center for Disease Control in Atlanta, Georgia, that a number of infants who were receiving a chloride deficient formula as their primary source of nourishment exhibited physical and behavioral abnormalities. These abnormalities consisted of weight loss, failure to grow, muscular weakness, delayed motor and speech development, chloride and potassium deficiencies, and a condition known as metabolic alkalosis in which the pH levels of the blood are excessively alkaline. To date, 141 documented cases of infants who were fed chloride deficient formulas have exhibited at least one episode of metabolic alkalosis. Analyses of these formulas have shown their chloride levels were about one-fifth of the amount recommended for infants by the American Academy of Pediatrics.

Once the formula was corrected there was remission of the metabolic abnormalities. The purpose of the research is to determine if there are any continuing complications or long-term sequelae associated with the past use of the chloride deficient infant formulas. This project is under the direction of Dr. Van Hubbard (NIH-NIAMDD) and consists of evaluations of the physical and psychological growth of these infants as well as evaluations of their biochemical, physiological, and neurological status, carried out at yearly intervals over several years. The Laboratory of Developmental Psychology is responsible for the psychological assessment of these children.

This study consists of two annual assessments of children who were fed these formulas and developed metabolic alkalosis. One assessment is at approximately 24 months of age and the second one at about 36 months of age. The first assessment has been completed and in the second assessment of these children is under way. In the first assessment the Bayley Scales of Infant Development were administered and the second assessment the McCarthy Scale of Intelligence was used. The McCarthy scales provide scores on memory, attention, language abilities, and motor skills--functions about which concern has been expressed. Siblings are tested on the follow-up assessment and serve as a control group. The two testings of the patient group provide the opportunity to determine if changes occur over time in the level of functioning of these children. The mothers are interviewed about their perception of the developmental status of the children, their anxieties and fears, and changes in their behavior and expectations concerning the child.

In the first assessment there was some indication that the amount of time that a child was exclusively on a chloride deficient diet was associated with lowered mental abilities.

Significance to Biomedical Research:

This is one of several studies in the Laboratory examining the consequences of deficits in early nutritional intakes for the behavioral development of the child. Here both the child's cognitive abilities and emotional characteristics are investigated.

Proposed Course:

Data collection will be completed by the fall of 1982. Analyses and reporting of data should be completed within the next six months.

Publications:

None

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|---|---|---------------------------------------|--------------------------|---------------------|----------|---------------------|---------------------|----------|--|--|--|----------------------|--------------------|------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02158-03 | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Impact of the Environment on the Development of the Abused Child | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Penelope K. Trickett</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LDP NIMH</td> </tr> <tr> <td>Elizabeth J. Susman</td> <td>Senior Staff Fellow</td> <td>LDP NIMH</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>OTHER: Ira S. Lourie</td> <td>Assistant Director</td> <td>DMHSP NIMH</td> </tr> </table> | | | PI: Penelope K. Trickett | Senior Staff Fellow | LDP NIMH | Elizabeth J. Susman | Senior Staff Fellow | LDP NIMH | | | | OTHER: Ira S. Lourie | Assistant Director | DMHSP NIMH |
| PI: Penelope K. Trickett | Senior Staff Fellow | LDP NIMH | | | | | | | | | | | | |
| Elizabeth J. Susman | Senior Staff Fellow | LDP NIMH | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| OTHER: Ira S. Lourie | Assistant Director | DMHSP NIMH | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Division of Mental Health Service Programs, NIMH Agencies and Institutions in the Washington Metropolitan area serving abusing families | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| TOTAL Staff Years: 3.30 | PROFESSIONAL: 1.30 | OTHER: 2.00 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This study focuses on the <u>emotional development of physically abused children</u> and the relationship between this development and the environment of the child. While clinical evidence shows that abused children are at risk for a wide range of physical and emotional problems, few controlled empirical studies exist and there is no research which relates aspects of the enduring environment of the abused child to the child's development. This study uses a <u>multi-method</u> <u>approach</u> to obtain information about the physical and psychological development of the abused child. These methods include observations, parent reports about daily interaction with their child, and level of parental frustration tolerance. The emotional development of the child is being assessed in relation to affec- tive coping, physical maturation, interpersonal problem solving, and peer relations. | | | | | | | | | | | | | | |

Project Description:

This study focuses on the development of physically abused children and the relationship between this development and the environment of the child. While there is wide agreement that the childhood victims of physical abuse are at risk for maladjustment, few controlled empirical studies bearing on this issue exist.

Research is needed to clarify the nature and intensity of the problems in the emotional development of abused children. It is also important to study the emotional development of abused children in the context of the rearing environment because such development is undoubtedly affected not just by the sporadic episodes of physical abuse but by the more enduring childrearing environment.

The present study is investigating aspects of the child rearing environment, specifically those processes which may lead to abusive incidents, and relates those processes to the emotional development of the child. There is evidence indicating that frequently the immediate antecedents of physical abuse of a child involve attempts at control of the child. However, the exact nature of this relationship is unclear. We are testing two competing hypotheses: (1) that abusive parents may believe that harsh physical punishment is a necessary technique if one is to rear a child adequately, or (2) that abusive parents, while not valuing physical punishment any more than other parents, tend toward out-of-control anger episodes which result from child misbehavior.

Subjects are physically abused children ranging in age from 4 to 10 years and their parents. They are recruited from local agencies serving this population. Criteria for inclusion in the sample include being two-parent families with both parents willing to participate in the study, and one of the parents being the abuser. Cooperating agencies contact families who fulfill these criteria, briefly explain the nature of the study and obtain a signed permission letter from those families who are willing to be contacted by research personnel for a more detailed description of the research. A control group of non-abusing families is recruited by advertisement. These families are matched to the abusing families on age, race, and sex of child and educational and occupational status of the parents. The total sample will include 60 families.

This study uses a multi-method approach to obtain information about the child-rearing environment of these families and the emotional development of the children. The families are first seen at the Laboratory of Developmental Psychology at which time the parents provide detailed information on the developmental history of the child and about their values, attitudes and reported practices of child discipline techniques. They fill out the Profile of Mood Scales, a measure of six mood states including anger-hostility and depression. Parents are also administered the Rothbart-Maccoby Role-Playing Task which presents parents with tape recorded typical child misbehavior incidents. They are asked to indicate what they would do if the incidents were to occur in their home. Parents also are asked to respond to the Block Q-sort of 92 items about child-rearing attitudes and values. Parents fill out the Family Environment Scale, a measure of the family psycho-social environment which includes such subscales as Cohesion, Control, Organization, and Achievement Orientation.

While the parents are taking part in these procedures, the child is, first, observed in a structured situation in order to assess his/her approach to new situations and a stranger, and then administered three tests: (a) the Peabody Picture Vocabulary Test, a standardized measure of receptive vocabulary from which a verbal IQ score is derived, (b) the Preschool or School Age Interpersonal Problem Solving Task, a measure of problem solving skill and (c) Bruininks-Oseretsky Test of Motor Proficiency (short form). After this, each parent, separately, is asked to work with his/her child on a structured task which requires cooperation between parent and child.

On a second visit to the laboratory, the interaction of the child with his/her parents and siblings is observed in semi-structured situations (such as playing, preparing and eating a snack).

One parent fills out a daily log for five consecutive days recording the misbehaviors of the child for the day and the discipline attempts provided by the parents.

Preliminary analyses suggest that, as measured by the Profile of Mood Scales, abusive mothers are more depressed than control mothers. This relationship does not hold for abusive and control fathers. Also, the parents of abused children report many more behavior problems in their children than do parents of control children. This is true for both the Internalizing and Externalizing Subscales of the Child Behavior Checklist.

Significance to Biomedical Research

This study addresses two distinct etiological issues. One focus is on the causes of child abuse with particular emphasis on the role played by parental psychopathology and parental child-rearing attitudes and behavior. The second focus is on the effect of child abuse on the psychological development of the victims. Information on these two issues can aid greatly in the development of treatment programs for abusive families and preventive interventions.

Proposed Course

Data collection is underway. More than half of the sample of families have been seen so far. Data collection and analysis will be completed in approximately two years.

Publications:

Trickett, P. K., Apfel, N. H., Rosenbaum, L. K., and Zigler, E. A five-year follow-up of participants in the Yale Child Welfare Research Program In Zigler, E. and Gordon, E. F. (Eds.), Day Care: Scientific and Social Policy Issues. Boston, Mass., Auburn House Publishing Co., 1981.

Zigler, E., Abelson, W. D., Trickett, P. K., and Seitz, V. Is an intervention program necessary in order to improve economically-disadvantaged children's IQ scores. Child Dev. 53: 340-348, 1982.

Trickett, E. J., Trickett, P. K., Castro, J. J., and Schaffner, P. The independent school experience: Aspects of the normative environments of single sex and co-ed secondary schools. J. Educ. Psychol., in press

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02159-02 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Information Processing and Adaptation to Research Hospitalization | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Elizabeth J. Susman Senior Staff Fellow LDP NIMH | | |
| OTHER: John C. Fletcher Special Assistant CC DIR | | |
| COOPERATING UNITS (if any) Office of the Director, Clinical Center | | |
| LAB/BRANCH Laboratory of Developmental Psychology SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: .45 | PROFESSIONAL: .20 | OTHER: .25 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Even after carefully executed <u>informed consent</u> procedures are carried out, patients may not fully process the nature and implications of the medical research in which they are participating. This study focuses on the <u>cognitive functioning</u> of patients in hospital-based clinical research. Participation in medical research is a stressful circumstance that provides a natural experiment in which patients' level of understanding and reasoning about personally stressful content (their illness and treatment regimens) is examined in relation to their cognitive functioning with regard to nonstress-related content and their level of anxiety. Participants include child, adolescent, and adult in-patients and normal volunteers at the NIH Clinical Center. Psychological assessments include standardized tests and interviews. | | |

Project Description:

Even after carefully executed informed consent procedures are carried out, patients may not fully process the nature and implications of the medical research in which they are participating. The purpose of this study is to examine cognitive functioning and adjustment of children, adolescents, and adults who are participants in hospital-based clinical research.

This study focuses on the cognitive functioning of patients in hospital-based clinical research. Participants are male and female children, adolescents, and adult patients at the NIH Clinical Center and comparison groups of normal volunteers. The second week after patients are admitted to the Clinical Center and six months later, they are administered a series of psychological assessments: (a) interviews designed to assess the patient's understanding of the causes of their illness and the nature of illness and treatment regimens, (b) standard tests of cognitive abilities and reasoning, and (c) the Spielberger-State-Trait Anxiety Scale.

Significance to Biomedical Research

This research has clinical significance for informed consent procedures and for the conduct of medical research. It has theoretical significance for understanding cognitive functioning in relation to conditions of psychological stress.

Proposed Course

The data will be collected over the next year. Data analysis will be carried out for one fiscal year and beyond after the data are collected.

Publications:

Susman, E. J., Nannis, E. D., Strobe, B. E., Hersh, S. P., Levine, A. S., and Pizzo, P. A.: Conceptions of cancer: The perspectives of children and their family. J. of Ped. Psychol., in press.

Blumberg, B. D., Lewis, J., and Susman, E. J. A time of transition In Eisenberg, M. G. and Jansen, M. A. (Eds.): Impact of Chronic Disabling Conditions on Self and Family. New York, Springer Press, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02160-02 LDP | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Development of Perspective Taking and Empathy in Early Childhood | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Ronald Iannotti</td> <td style="width: 35%;">Guest Worker</td> <td style="width: 15%;">LDP</td> <td style="width: 10%;">NIMH</td> </tr> <tr> <td colspan="5" style="height: 20px;"></td> </tr> <tr> <td>Other:</td> <td>Mark Cummings</td> <td>Staff Fellow</td> <td>LDP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Carolyn Zahn-Waxler</td> <td>Research Psychologist</td> <td>LDP</td> <td>NIMH</td> </tr> </table> | | | PI: | Ronald Iannotti | Guest Worker | LDP | NIMH | | | | | | Other: | Mark Cummings | Staff Fellow | LDP | NIMH | | Carolyn Zahn-Waxler | Research Psychologist | LDP | NIMH |
| PI: | Ronald Iannotti | Guest Worker | LDP | NIMH | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | |
| Other: | Mark Cummings | Staff Fellow | LDP | NIMH | | | | | | | | | | | | | | | | | | |
| | Carolyn Zahn-Waxler | Research Psychologist | LDP | NIMH | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL Staff Years: .00 | PROFESSIONAL: .00 | OTHER: .00 | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The Principal Investigator on this project is not currently employed in the Laboratory. Data already collected from analyses in this project will be incorporated in Z01 MH 02146-03. | | | | | | | | | | | | | | | | | | | | | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02161-02 LDP | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Developmental Changes in Imitative Learning | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Leon Kuczynski</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">LDP NIMH</td> </tr> <tr> <td>Other: Carolyn Zahn-Waxler</td> <td>Research Psychologist</td> <td>LDP NIMH</td> </tr> <tr> <td>Marian Radke-Yarrow</td> <td>Chief</td> <td>LDP NIMH</td> </tr> </table> | | | PI: Leon Kuczynski | Visiting Fellow | LDP NIMH | Other: Carolyn Zahn-Waxler | Research Psychologist | LDP NIMH | Marian Radke-Yarrow | Chief | LDP NIMH |
| PI: Leon Kuczynski | Visiting Fellow | LDP NIMH | | | | | | | | | |
| Other: Carolyn Zahn-Waxler | Research Psychologist | LDP NIMH | | | | | | | | | |
| Marian Radke-Yarrow | Chief | LDP NIMH | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | | | | |
| SECTION | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | |
| TOTAL Staff Years: .26 | PROFESSIONAL: .16 | OTHER: .10 | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This study is concerned with the development of children's <u>imitative behavior</u> <u>in natural settings</u> . Data were obtained on 40 children over a nine month period during the second and third years of life. Sources of data consisted of descriptive accounts of imitation by mothers trained in observational recording. Mothers' records included children's immediate and delayed imitations. Frequently occurring categories of imitation included nonverbal expressions of emotion, and behaviors involved in parental nurturance, care taking, working, and discipline. The nature of the children's imitation will be examined in relation to personality variables of the children and the mothers. | | | | | | | | | | | |

Project Description:

This study is concerned with the early development of imitative behavior of young children in the natural setting. Children's imitation of the behaviors of parents, siblings, and models outside the home has been considered to be an important process in children's acquisition of complex patterns of behavior. Previous research on imitation has tended to use experimental paradigms in which preschool and school-age children, whose imitative repertoires are well past the acquisition stage, are studied. Further, the experimental research on imitation has focused on variables such as characteristics of the model and the situation that govern the acquisition of imitation. The extent to which findings are valid and representative is open to question because information is lacking regarding both the early acquisition and the basic repertoires of imitation that children bring to the experimental setting. Information about the content of behaviors that are spontaneously imitated by children may begin to clarify the processes to which learning by observation contributes. Experimental research has also emphasized the study of immediate rather than delayed imitation. Although delayed imitation, imitation that occurs some time after the modeled behavior, is more difficult to investigate than immediate imitation, it may also provide a better index of long-term patterns of behavior acquired through the process of observing models.

The present study extends previous research by investigating the development of both immediate and delayed imitation as it occurs in natural settings. One source of data was detailed narrative accounts of children's behaviors recorded by mothers trained in observational procedures. Reliabilities of maternal reports were assessed by comparing mothers and investigators' reports of children's imitations during home visit (percent of agreement was 91%). Mothers were also interviewed every three weeks during the data collection period and were asked to report new forms of imitation that occurred since the preceding contact. Data were obtained on 40 children covering a nine-month period in the second and third years of life.

Preliminary analyses indicate that during the second year of life imitative repertoires are both extensive and increasingly complex. Infrequent yet salient behaviors such as characteristic expressions of emotion and idiosyncratic behaviors of models comprise a large category of immediate imitation. Complex patterns of behavior involved in the performance of household chores, grooming, and caretaking, as well as parental verbalizations during disciplinary interactions are a source of both immediate and delayed forms of imitation. Children's imitation of aspects of parental discipline implicates imitation in the development of conscience and self-control.

Significance to Biomedical Research:

Imitation is a basic process of learning and has obvious implications for the environmental transmission of complex patterns of behavior, including patterns of disordered behavior. This study focuses on the learning of very young children using the parents as models of behavior and affective expression. Although few studies have investigated what behaviors are susceptible to imitation, disordered forms of parental behavior may, in part, be transmitted by this process. This study makes a start at examining the content of imitated behaviors during the second year of life.

Proposed Course:

The data for this study have been collected. Data analysis and preparation for publication of the study should be completed within a year's time.

Publications:

None

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|---|---|---|--------------------|--------------|----------|----------------------------|---------------------------|--------------------------------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02162-02 LDP | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Patterns of Psychological Functioning and Psychosexual Identity in Children with Congenital Adrenal Hyperplasia | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Jerome H. Blue</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LDP NIMH</td> </tr> <tr> <td>OTHER: Arnold Slyper, M.D.</td> <td>Pediatric Endocrinologist</td> <td>Children's Hosp. Washington, D.C.</td> </tr> </table> | | | PI: Jerome H. Blue | Staff Fellow | LDP NIMH | OTHER: Arnold Slyper, M.D. | Pediatric Endocrinologist | Children's Hosp. Washington, D.C. |
| PI: Jerome H. Blue | Staff Fellow | LDP NIMH | | | | | | |
| OTHER: Arnold Slyper, M.D. | Pediatric Endocrinologist | Children's Hosp. Washington, D.C. | | | | | | |
| COOPERATING UNITS (if any) Children's Hospital National Medical Center Washington, D.C. | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL Staff Years: .00 | PROFESSIONAL: .00 | OTHER: .00 | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | |
| This project has been discontinued. | | | | | | | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH-02163-02 | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Psychobiological Correlates of Behavior Problems | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Jerome H. Blue</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LDP NIMH</td> </tr> <tr> <td>OTHER: Sarah H. Broman</td> <td>Acting Chief, Mental Retardation and Learning Disorders Sec.</td> <td>DNB NINCDS</td> </tr> </table> | | | PI: Jerome H. Blue | Staff Fellow | LDP NIMH | OTHER: Sarah H. Broman | Acting Chief, Mental Retardation and Learning Disorders Sec. | DNB NINCDS |
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| COOPERATING UNITS (if any) National Institute of Neurological and Communicative Disorders and Stroke | | | | | | | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL Staff Years: | PROFESSIONAL: | OTHER: | | | | | | |
| 40 | 20 | 20 | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | |
| <p>Children with congenital malformations are studied in relation to specific behavior problems and patterns of cognitive abilities. Data on major and minor <u>congenital malformations</u>, patterns of cognitive abilities and childhood disorders from the <u>Perinatal Collaborative Project</u> are examined. This systematic analysis of <u>congenital malformations</u> and <u>patterns of congenital abilities</u> which might be related to various behavior problems has only recently started. Preliminary analyses suggest that there are basic brain and behavior relationships. Particular types of congenital malformations were highly correlated with specific behavior disorders.</p> | | | | | | | | |

Project Description:

Some studies have shown behavior disorders to occur more frequently in children with particular malformations, while other studies have shown several factors to predict childhood disorders. Most analyses of childhood disorders have focused on either cognitive, behavioral, or physical characteristics and not an interaction between these factors. Few diagnostic categories other than hyperactivity have been analyzed and we stress that there is a need to look at correlates of other behaviors as well. In addition, few studies have examined a large number of malformations using a substantial sample of children with behavior disorders. Cognitive and biological correlates of various behavior problems using a large sample will be investigated in order to better understand maladjustment.

Children with congenital malformations are being studied in relation to specific behavior problems that are most critical or resistant to change, like fighting, impulsivity, shyness, and others. Because congenital malformations may interfere with the acquisition of many skills and the behavioral development of children it is important to determine whether children with such abnormalities are likely to need special attention. Thus, this project is designed (1) to document the cognitive, behavioral, and congenital malformations of children with special problems, and (2) specify the interactions among these factors in childhood disorders.

Data from the Perinatal Collaborative Study which includes neurological examinations, behavior profiles, and a variety of psychological tests will be used. The neurological examinations were used to classify the major and minor congenital malformations of these children. The behavior profiles consist of a variety of problems which have been correlated with childhood disorder or psychopathology (i.e., Fearfulness, Emotional reactivity, Non-communication, Thumb-sucking, Nail biting, etc.). The measures of cognitive abilities include subtests of the Wechsler Intelligence Scale for Children and other psychological tests.

Preliminary data analysis shows that many children diagnosed with a particular malformation at birth were not comparably diagnosed 7 years later, and other children showed signs of a malformation at the 7 year but not at the perinatal examination. Children diagnosed with either Syndactyly or Deformed ear pinna at birth and 7 years later have several behavior problems with high stability coefficients. However, none of the behaviors with high stability coefficients was the same in both groups. Children with no noticeable malformation at birth but diagnosed 7 years later have behavior problems somewhat consistent with children who were given the same diagnosis at both examinations.

Significance to Biomedical Research:

This study has the potential for delineating relationships between a variety of child behavior disorders and congenital malformations.

Z01 MH-02163-02

Proposed Course:

Data analyses have begun.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02164-02 LDP |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Impact of Biological Changes on Psychological Functioning during Adolescence | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| CO PI: Elizabeth J. Susman Editha Nottelmann Jerome H. Blue | Senior Staff Fellow Staff Fellow Staff Fellow | LDP NIMH LDP NIMH LDP NIMH |
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| COOPERATING UNITS (if any) Office of the Director, Clinical Center Developmental Endocrinology Branch | | |
| LAB/BRANCH Laboratory of Developmental Psychology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL Staff Years: 2.10 | PROFESSIONAL: .60 | OTHER: 1.50 |
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| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This research examines <u>biological and psychological relationships</u> during <u>adolescence</u> . Children and adolescents are studied through the pubertal period. Participants are male and female 9- to 14-year-olds and their parents. At three times of measurement, six months apart, participants are evaluated for stage of <u>pubertal development</u> by blood samples for <u>gonadotropins</u> , <u>gonadal steroids</u> , and <u>adrenal androgens</u> as well as by a physical examination to determine their Tanner stage. Psychological assessments include standardized psychological tests and systematic <u>observation of parent-child interactions</u> . | | |

Project Description

The theoretical framework for the study is multidisciplinary. Viewed from the perspectives of psychology, psychiatry, and biology, adolescence is recognized as a period characterized by rapid and pervasive changes. Changes in endocrine functioning and the external manifestation of these changes, physical growth, and changes in cognitive, emotional, and social development constitute pervasive experiences during adolescence.

Although there is evidence from studies of adult humans and subhuman primates indicating links between endocrine changes and behavioral and psychological functioning, empirical study of the relationship between hormone levels and behavior in normal or disturbed adolescents is limited.

A wide range of psychological problems shows dramatic increases during adolescence. These problems include serious psychiatric disturbances (such as unmanageable anxiety, hostility, and depression) as well as mood fluctuations, lack of impulse control, social isolation, and poor self concept. There are also marked changes in cognitive properties and in interests. The links between these behaviors and biological and environmental factors are the focus of study.

Participants in the study are black and white, lower-middle to upper-middle class, 9- to 13-year-old girls and 10- to 14-year-old boys and their parents, recruited primarily from church and secular groups. Eighty participants will be recruited for the study, an equal number of males and females in each of the five Tanner stages of puberty. At three times of measurement, six months apart, the participants are evaluated on biological and psychological measures.

Biological measures include measures of external physical development and endocrine assessments: height, weight, head circumference, Tanner staging, and blood levels of gonadotropins, gonadal steroids, and adrenal androgens. Parents and adolescents provide reports of physical development and change. Of special interest are the variables of age at onset of puberty and rate of maturational changes. Previous studies of the relation between physical development and psychological variables have suffered from imprecise or incomplete evaluations of stage of puberty. Including both external physical measures and endocrine assessments may reveal that the two sets of measures are predictive of different psychological variables. The biological data are collected either as part of this protocol, or as part of "The Relationship of Adrenarche and Gonadarche in Normal and Precocious Puberty" and/or "Basal and Stimulated Gonadotropins and Gonadal Steroids in Normal Volunteers from 10-15 Years of Age" (Protocols #80-CH-32 and 80-CH-160, respectively) conducted by the Developmental Endocrinology Branch, NICHD, if a participant chooses to be in both the NIMH and an NICHD protocol.

Initially, a psychiatric evaluation is made of the adolescents and their parents. Each parent provides a development history of the adolescent which includes a health history and assessment of child-rearing attitudes toward adolescents and attitudes about their own adolescence. At each of the three periods of measurement, within five days prior to the biological assessments, psychological assessments are made.

To investigate relations between rate of maturation and verbal and spatial cognitive abilities, the participants are administered the Information and Block Design subtests of the Wechsler Intelligence Scale for Children, and the Verbal Reasoning, Spatial Relations, and Perceptual Speed subtests of the Primary Mental Abilities Test.

There are multiple measures of the adolescent's emotional and social behavior: Parents provide detailed reports of the adolescent's moods and interpersonal behavior. Checklists describing the content and intensity of the adolescent's moods and social behaviors also are completed daily by the adolescents and their mothers during the week following laboratory data collection procedures. Psychological problems are assessed using the Achenbach Child Behavior Checklist, and the Offer Self-Image Questionnaire for Adolescents. Other standardized tests of self-assessment include a perceived competence scale, a personal attributes questionnaire, a test of gender-role identity; and a measure of interpersonal reasoning in social situations. During the laboratory visit, parents and adolescent work together as triads and dyads on conflict-resolution tasks (on how to handle family problems, and personality traits of the adolescent on which there is known disagreement between parent and child). The interactions are videotaped and coded for variables hypothesized to be related to hormone levels.

Significance to Biomedical Research:

This research has significance for understanding relationships between developmental endocrinological changes and behavioral characteristics of the adolescents. It will contribute also to an understanding of psychiatric disturbances during early adolescence. Such information is relevant to prevention and treatment programs concerning problems of adolescents.

Proposed Course:

Data analysis will continue for approximately two more years. Data analysis on the cross-sectional aspects of the study will begin in the coming year. Data analysis will continue on the longitudinal aspect of the study after data collection is completed.

Publications:

None

PROJECT DESCRIPTION:

The objective of the studies in this project is to delineate the neural system underlying memory formation in the monkey and to differentiate it from the neural system that underlies habit formation. The methods used include behavioral analyses of the effects of selective cerebral ablations and disconnections, anatomical analyses of functional neural pathways, and both behavioral and anatomical developmental analyses. The studies are based directly on information derived from the other projects in this laboratory, all of which deal with various aspects of stimulus processing and encoding. The results from these other projects suggest that the sensory system for each modality is composed of two hierarchically organized corticocortical pathways, one directed ventrally to the temporal-lobe limbic system and concerned with object perception, the other directed dorsally to the frontal-lobe motor system and concerned with spatial perception. The ultimate goal of this project is to determine how object and spatial perceptions in the different modalities are formed into memories, how these different memories are associated with each other, how they evoke emotions and motor acts, and how they lead not only to these cognitive events but also to habit formation. Our progress in understanding each of these processes will be described in turn.

(1) Recognition memory:

Previous studies suggested that one-trial object recognition (delayed nonmatching-to-sample with trial-unique objects) depends on a reciprocal cortico-limbo-thalamic pathway that leads to the storage of the encoded representation of the stimulus in anterior temporo-insular cortex. New studies have shown that this pathway actually contains two relatively independent limbo-thalamic segments, one from the amygdala through the amygdalofugal pathways (AFP) to the magnocellular portion of n. medialis dorsalis (MDmc), and the other from the hippocampus through the fornix (Fx) to the anterior thalamic nuclei (Ant N). The evidence is based on comparison of the effects of separate and combined AFP and Fx transections, as well as of separate and combined MDmc and Ant N ablations. In both cases, the combined lesions yielded significantly greater recognition losses than did the separate lesions. Because of their newly discovered functional significance, the two limbo-thalamic projection systems are now being mapped in detail with axonal transport techniques. The initial results show that the amygdaloid projections arise throughout the complex, though most heavily from the basomedial nucleus, sweep through the substantia innominata, and then travel in the inferior thalamic peduncle to enter the head of the thalamus before passing caudally to terminate in MDmc and n. reuniens; allocortical areas adjacent to the amygdala also contribute significantly to this projection. Hippocampal projections arise predominantly in the subiculum and terminate most heavily in nuclei anterior medialis, anterior ventralis, and lateralis dorsalis, with lighter projections to nuclei reuniens, centralis latocellularis, rotundis, and paraventricularis; all of these projections course through the medial part of the fornix, though n. lateralis dorsalis also receives a nonfornical input which runs through the medial pulvinar. New studies are being undertaken to look at the effects on recognition memory of damage to other diencephalic targets of the limbic system, such as the

mamillary bodies, as well as of damage to the prefrontal cortical targets of MDmc and Ant N.

(2) Associative memory:

Like their efferent pathways and thalamic targets, the amygdala and hippocampus make approximately equal contributions to recognition memory. In the case of associative memory, however, new results indicate that these two limbic structures make very different contributions. In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two visual stimuli after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, but the amygdalectomized monkeys fell to chance performance. Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance. The results of these two complementary experiments indicate that although both the amygdala and hippocampus are important for associative memory, their roles are totally different. Many further analyses along the lines of these two experiments are needed, however, before the selective associative memory functions of the amygdala and hippocampus can be precisely identified. For example, the association of an object with an affective state, such as fear, pleasure, etc. appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view is being obtained in an experiment showing that one-trial object-reward association is impaired far more by amygdaloid than by hippocampal lesions. By contrast, because of the contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

(3) Habit Formation:

On all of the memory tasks described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, animals with the combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours.

Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals. This paradoxical success in the presence of severe memory loss implies the existence of an important retention mechanism outside the limbic structures of the temporal lobe.

We have since performed additional experiments to characterize further the essential difference in function between the limbic and nonlimbic retention mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of retention from memory, we have labelled it 'habit formation'. Further investigation of this mechanism of habit formation as well as elucidation of its neural substrate have become important goals of our research.

On the evidence that in the adult monkey there may be two relatively independent systems for retention of information, we recently initiated a series of studies to assess the development of these two systems in infant monkeys. Results thus far indicate that one-trial recognition, requiring memory of one object at a time for only 10 sec each, is absent in infants younger than four months of age and does not reach adult levels of proficiency even at one year. This slow ontogenetic development of recognition memory was shown even more strikingly with longer delays and lists. In sharp contrast, when 3-month-old infant monkeys were trained on object discrimination habits, they performed exactly like adult monkeys in both acquisition and retention, even though intertrial intervals lasted 24 hours. These results strongly suggest that the two systems of retention that were found to be relatively independent in the adult monkey are also developmentally dissociable. Indeed, they provide evidence that the mysterious phenomenon of infantile amnesia could be due to the absence of a functional memory system in early childhood. On the basis of this evidence, we have begun to prepare monkeys with neonatal removal of this system (i.e. combined amygdalo-hippocampal removals) in an attempt to see how cognitive, emotional, and social behavior develops in animals whose amnesia persists from infancy through adulthood. This study will help to evaluate two provocative proposals from the clinical literature, namely, (a) that early dysfunction of the limbo-thalamic memory system could be one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology, and (b) that the reason a pure case of amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clinical picture of an amnesic adult.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

In the process of investigating the role of various temporal-lobe structures in the visual memory of the monkey, we obtained a result that is particularly exciting because it appears to solve the long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in monkeys by removal of the hippocampus alone have largely failed. What we have found in our studies, both for recognition memory and for object-reward association memory, is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss does ensue. The discovery has not only resolved the discrepancy between clinical and animal findings but has also provided new insight into the neural substrate of memory. Specifically, it has led to the development of a hierarchical model of recognition and associative memory involving a reciprocal cortico-limbo-thalamic memory circuit that may well serve as the foundation for all cognitive processes beyond perception, including thought. As we gain further understanding of the memory system, and how it differs from the noncognitive system for habit formation, we will inevitably gain a better understanding of thought and its breakdown in normal and abnormal behavior.

PROPOSED COURSE OF RESEARCH:

Since the two major thalamic structures implicated in the memory system, namely, the magnocellular portion of n. medialis dorsalis and the anterior thalamic nuclei, are known to send heavy projections to adjacent cortical fields in the medial part of the frontal lobes, we shall look next at the effects on memory of damaging these fields to determine whether they comprise a prefrontal extension of the memory system, and, if so, what their selective contributions might be. In addition, we shall continue our attempts to differentiate between amygdaloid and hippocampal contributions to associative memory and test whether the distinction is carried further through the thalamic and prefrontal segments of the circuit. We shall also initiate studies to explore the neural basis of habit formation, with the cortico-striatal projection system as our initial target. Finally, we expect to continue our examination of the effects of neonatal limbic lesions on social and emotional behavior as well as on memory and learning, to test whether such a preparation does indeed provide an animal model of childhood autism.

PUBLICATIONS:

Spiegler, B.J., and Mishkin, M.: Evidence for the sequential participation of inferior temporal cortex and amygdala in the acquisition of stimulus-reward associations. Behav. Brain Research 3: 303-317, 1981.

Mishkin, M., and Aggleton, J.: Multiple functional contributions of the amygdala in the monkey. In Ben-Ari, Y. (Ed.): The Amygdaloid Complex. Elsevier/North-Holland Biomedical Press, 1981, pp. 409-420.

Mishkin, M., Spiegler, B.J., Saunders, R.C., and Malamut, B.L. An animal model of global amnesia. In Corkin, S. et al. (Ed.): Alzheimer's Disease: A Report of Progress (Aging, Vol. 19). Raven Press, New York, 1982, pp. 235-247.

Mishkin, M.: A memory system in the monkey. Phil. Trans Royal Soc. Lond. (B), 298: 85-95, 1982.

Zola-Morgan, S., Squire, L.R., and Mishkin, M.: The neuroanatomy of amnesia: amygdala-hippocampus vs temporal stem. Science (in press) 1982.

Mishkin, M., Lewis, M.E., and Ungerleider, L.G.: Equivalence of parieto-preoccipital subareas for visuospatial ability in monkeys. Behav. Brain Research (in press) 1982.

Mishkin, M. and Ungerleider, L.G.: Contributions of striate inputs to the visuospatial functions of parieto-preoccipital cortex in monkeys. Behav. Brain. Research (in press) 1982.

PROJECT DESCRIPTION:

Objectives:

Ablation studies have shown that inferior temporal cortex is critical for the performance of pattern discrimination and pattern recognition tasks in primates. Furthermore, single-unit recording studies of the anterior part of inferior temporal cortex (area TE) over the past 10-12 years have shown that many of the visually responsive neurons in this area are strongly activated by visual stimuli with complex features, e.g. a bottle brush, a hand, a face, etc. These recording studies, which were largely carried out in lightly anaesthetized, paralyzed monkeys, demonstrated not only that area TE cells are sensitive to particular patterns, but also that the receptive fields of the cells all cover the central part of the visual field, with greatest sensitivity occurring at the center of gaze, or fovea. Also, the receptive fields were found to be large and to extend into both the contralateral and ipsilateral visual fields.

Other studies, which were carried out in awake monkeys performing visual tasks, have shown not only that the pattern of the stimulus influences the responsiveness of the single neurons, but also that the part the pattern plays in the task is influential. For example, when a pattern is shown to a monkey so that he can later indicate whether a second pattern is the same as the first or not - delayed matching-to-sample - the neuronal response to the pattern is often weak or absent during the first presentation but strong when the pattern is presented the second time. In order to specify the visual information to which inferior temporal neurons are sensitive, it is first necessary to understand and control these behavioral and attentional influences on inferior temporal neurons, and it is these influences to which our initial experiments have been directed.

In order to carry out the experiments, a flexible and powerful on-line computer software system has been developed for the control of the behavioral paradigms and for gathering single-unit, event, and analog data at high resolution with a laboratory minicomputer. This technical development was carried out in collaboration with members of the Laboratory of Sensorimotor Research of the National Eye Institute. The system is currently being used to record from single neurons in area TE while the animal fixates one stimulus and neuronal responsivity is examined with other visual stimuli. Currently, three other laboratories at the NIH have adopted this system and one at Johns Hopkins has adopted a modified version. In addition, several other laboratories are in the process of switching over to use of the system.

Major findings:

So far we have recorded from over 450 cells distributed throughout area TE of 5 different monkeys. In our initial experiments we unexpectedly found inconsistent neuronal responses to large spots and squares of light presented as probe stimuli while the monkey was fixating a smaller spot of light - the fixation spot - in order to detect the dimming of this spot and thereby obtain a reward. Previous studies of inferior temporal neurons, whether carried out

in anesthetized or awake monkeys, used no fixation spot. In order to overcome any possible influence of the fixation spot, we turned it off for a short period (1000 msec). If a probe stimulus was presented during this "blink" of the fixation spot, about 60% of the cells encountered gave a highly consistent response. The animal's eye position was monitored automatically, and if the eye moved more than 1 degree in any direction the trial was discontinued and the animal had to initiate a new trial. By changing stimulus parameters and stimulus positions, the following results were obtained. In most visually responsive inferior temporal cells, the fixation spot exerts a very potent influence, more potent even than changes in the features of the probe stimulus (although in these experiments only the size and relative proportions of slits and squares were varied). When the same stimulus that caused a neuronal response during the "blink" was presented while the fixation spot remained on, the response was either much weaker or disappeared completely. As a result, the size of the receptive field became much smaller when the fixation spot was present than when it was absent. Under both conditions, however, the best response always occurred when the stimulus was presented at the center of gaze, i.e., projected onto the fovea. The response latencies to stimulus onset were between 70 and 200 msec. These and other characteristics of the neuronal responses to the stimulus during the "blink" of the fixation spot were similar to responses obtained previously by others in the lightly anesthetized, immobilized preparation.

In order to determine if increased attention to the stimulus during the "blink" of the fixation spot might be causing the increased strength and consistency of response, we altered the task so that the monkey, while still fixating, was required to respond to the dimming of the probe stimulus rather than of the fixation spot. When the monkey responds correctly in this condition, we can assume his attention must be directed to the stimulus. These experiments were carried out in both the "blink" situation (fixation spot absent) and the fixation situation (fixation spot present). In both cases, the neuronal response to the stimulus was weaker than before. Indeed in the task in which the monkey responded to the stimulus dimming and the fixation spot remained present, the neuronal response was weakest of all, often to the point of no response, showing that the suppression due to the fixation point and suppression due to attention are additive. Thus, responses of inferior temporal neurons to visual stimuli are suppressed simply by the physical presence of the fixation spot. Furthermore, the improvement of response when the fixation spot is absent can not be due to a shift in spatial attention to the probe stimulus, because in tasks where such a shift in spatial attention was explicitly required the strength of the response was actually weakened.

This suppressive effect of attention, however, was obtained in behavioral tasks in which the specific pattern of the stimulus was not relevant for correct performance. Since pattern discrimination depends so critically on the integrity of the inferior temporal cortex, we modified the behavioral tasks so that we could compare the results of the foregoing experiments with those obtained when the pattern of the stimulus was relevant for correct performance. In the modified tasks the monkey was required to distinguish between a simple pattern, such as a plus sign, and the square or slit that had

been presented in the previous experiments. The behavioral response to the plus sign was an immediate lever release, while the behavioral response to the probe stimulus, i.e. the square or slit, was to release the lever only when it dimmed. Thus, the behavioral response to the probe stimulus was in every way the same as it had been in the previous experiments except that the monkey now had to distinguish the shape of the stimulus when it first appeared in order to make the correct response. As in previous tasks, the retinal locus of the stimulus was closely controlled by the requirement that the monkey maintain constant eye position. Many neurons now gave a strong response to the probe stimulus even when the fixation spot (previously a potent suppressive influence) was present. Under these conditions, the strength of response approached or even surpassed that seen when neither suppressive influence was present (i.e. the blink task).

These results show for the first time (a) that attention to the spatial location or intensity of a stimulus or both are not the only attentional factors that influence the responses of single neurons, and (b) that the responses of many inferior temporal neurons may be markedly altered depending on which stimulus feature the monkey is currently attending to. Indeed attention to different stimulus variables can have opposite influences on the response of the same neuron.

In summary, we have demonstrated that inferior temporal neurons show reduced responsivity to visual stimuli and, perhaps because of this, reduced receptive field size under the suppressive influence of either or both a fixation spot and attention to the position or the luminance of the probe stimulus. By contrast, when the animal is attending to the pattern of the visual stimulus, because this feature has become important for behavioral decisions, the neuronal response to the stimulus is dramatically enhanced.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

Solving the problem of how the brain processes sensory information has been a major goal of psychologists and neurophysiologists. These experiments are an attempt to move closer to an understanding of such processing in the visual system of the primate. Since the inferior temporal region is involved in higher-order visual processing, we expect that our investigations will not only further our knowledge of how this cortex codes and stores visual stimuli but will also provide insight into visual perceptual and memory problems seen clinically as a result of acquired or congenital defects.

PROPOSED COURSE OF RESEARCH:

Experiments on the role of differing types of attention on the responses of single cells will be continued. However, now that some of these attentional influences have been measured and analyzed they can be taken into account and new studies initiated which will focus more on the sensitivity of inferior temporal neurons to changes in stimulus pattern. Such study requires the use of sets of stimuli that have some orderly interrelationship. Many sets of

stimuli meet this requirement of orderliness, but the one we have chosen to begin with is the set of sine-wave spatial frequency gratings. These have the advantages of being well known mathematically, easy to produce, and, with appropriate superposition, able to reproduce any two-dimensional visual scene. Furthermore, psychophysical experiments have suggested that the human visual system acts as a small number of spatial frequency channels or filters, each one of which is analyzing images for the amount of that particular spatial frequency or sine wave. Finally, psychophysicists have proposed that the visual system can analyze patterns by determining the ratios of the different spatial frequencies they contain. The hypothesis is elegant, and experiments we are planning to test it should yield valuable data even if the hypothesis proves to be incorrect.

In addition, an effort will be made to correlate single-cell results with behavioral results in normal and operated monkeys, as well as with psychophysical results we hope to gather in normal human subjects and patients with brain lesions. Finally, if time and personnel permit, recording of single neurons in parts of the amygdala that receive the major inferior temporal output will be undertaken in an attempt to try to determine what transformation in visual information has taken place at this next station in the visual pathway.

PUBLICATIONS

Richmond, B. J. and Wurtz, R. H. 1982. Inferotemporal cortex in awake monkey. In: Morrison, A. R. and Strick, P. L. (eds.), Changing Concepts of the Nervous System. Academic Press, pp. 411-422.

Hays, A.V., Jr., Richmond, B.J., and Optican, L.M. 1982. A UNIX-based multiple-process system for real-time data acquisition and control. Wescon Conference Proc., pp. 163-174.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02033-05 LN | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Functional mapping of sensory systems | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: K.A. Macko</td> <td style="width: 40%;">Staff Fellow</td> <td style="width: 30%;">LN NIMH</td> </tr> <tr> <td>OTHER: M. Mishkin</td> <td>Act. Chief, Laboratory of Neuropsychology</td> <td>LN NIMH</td> </tr> <tr> <td>J. Bachevalier</td> <td>Quebec Council for Health Res. Fellowship</td> <td>LN NIMH</td> </tr> <tr> <td>C. Kennedy</td> <td>Guest Worker</td> <td>LCM NIMH</td> </tr> <tr> <td>L. Sokoloff</td> <td>Chief, Laboratory of Cerebral Metabolism</td> <td>LCM NIMH</td> </tr> <tr> <td>R.K. Nakamura</td> <td>Senior Staff Fellow</td> <td>LPP NIMH</td> </tr> </table> | | | PI: K.A. Macko | Staff Fellow | LN NIMH | OTHER: M. Mishkin | Act. Chief, Laboratory of Neuropsychology | LN NIMH | J. Bachevalier | Quebec Council for Health Res. Fellowship | LN NIMH | C. Kennedy | Guest Worker | LCM NIMH | L. Sokoloff | Chief, Laboratory of Cerebral Metabolism | LCM NIMH | R.K. Nakamura | Senior Staff Fellow | LPP NIMH |
| PI: K.A. Macko | Staff Fellow | LN NIMH | | | | | | | | | | | | | | | | | | |
| OTHER: M. Mishkin | Act. Chief, Laboratory of Neuropsychology | LN NIMH | | | | | | | | | | | | | | | | | | |
| J. Bachevalier | Quebec Council for Health Res. Fellowship | LN NIMH | | | | | | | | | | | | | | | | | | |
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| L. Sokoloff | Chief, Laboratory of Cerebral Metabolism | LCM NIMH | | | | | | | | | | | | | | | | | | |
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| COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, NIMH; Laboratory of Psychology and Psychopathology, NIMH | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neuropsychology | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS X | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The [¹⁴ C] <u>2-deoxyglucose method</u> was used to identify the cerebral areas related to vision through a comparison of glucose utilization in a visually stimulated as compared to a visually deafferented hemisphere in the <u>rhesus monkey</u> . Cortically, the visually related areas included the entire expanse of <u>striate</u> , <u>prestriate</u> , and <u>inferior temporal cortex</u> as far forward as the temporal pole, the posterior part of the <u>inferior parietal lobule</u> , and the <u>prearcuate</u> and <u>inferior prefrontal cortex</u> ; subcortically, in addition to the lateral geniculate nucleus and superior colliculus, visually related structures included large parts of the <u>pulvinar</u> , <u>caudate</u> , <u>putamen</u> , <u>claustrum</u> , and <u>amygdala</u> . These results, which are consonant with a model that postulates an occipito-temporo-prefrontal pathway for <u>object vision</u> and an occipito-parieto-prefrontal pathway for <u>spatial vision</u> , reveal the full extent of those pathways and localize their points of contact with limbic, striatal, and diencephalic structures. | | | | | | | | | | | | | | | | | | | | |

PROJECT DESCRIPTION:

Converging evidence from studies of lesion effects, anatomy, and single units indicates that, in the primate, the system for processing information about visual objects extends beyond the striate cortex to include the circumstriate and inferior temporal areas. From the inferior temporal area, in turn, information about these objects appears to be transmitted to subcortical structures in the temporal lobe and to inferior prefrontal cortex. The same classical mapping techniques have also been used to identify another system, specialized for processing information about spatial vision, which also depends on a corticocortical pathway, in this case from striate through prestriate to inferior parietal and dorsal prefrontal cortex. In addition, both systems are known to have subcortical inputs to, and projections from, structures in the tectofugal pathway. Despite a range of research efforts, however, the full extent and precise boundaries of these cortical pathways are still undefined, as are their exact points of contact with their subcortical forebrain targets. The [^{14}C] 2-deoxyglucose method for measuring local cerebral glucose utilization (LCGU) has provided a means of clarifying these issues.

Experiment 1

To map the functional visual system by means of the 2-deoxyglucose technique, we prepared monkeys with a unilateral optic tract section alone or a tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare LCGU values in a "seeing" and a "blind" hemisphere within the same animal and thereby map the visually related areas.

Monkeys in this initial study sat in a primate chair while visual patterns mounted on a drum rotated around their heads. They were injected with a pulse of radioactively labeled 2-deoxyglucose and monitored over the next 45 minutes to determine the levels of free deoxyglucose in the blood. Most of the radioactively labeled substance is incorporated into active cells during this period, and any extracellular deoxyglucose is of sufficiently low concentration that its presence does not contaminate the autoradiographs.

In a second study, monkeys prepared surgically as above were trained on a visual pattern discrimination task in which they were required to respond with the hand opposite the blind hemisphere. In this task a positive stimulus was paired with one of a series of negative stimuli in sequential blocks of trials, and correct responses were reinforced with a water reward.

In both behavioral situations, reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex and reaching even to the inferior prefrontal cortex. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe, side-to-side differences were seen in lateral and dorsal amygdala, ventral putamen, ventral claustrum, and tail of caudate. Outside this

stimulus-processing system specialized for object vision, asymmetries were also seen in the inferior parietal lobule and prearcuate region of the frontal lobes, the tissue specialized for spatial vision. Performance on the discrimination task led to an asymmetrical increase of glucose utilization in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking. In the cortical tissue related to vision the effects of the two different behavioral situations were substantially the same. In the subcortical tissue related to vision, however, the monkeys performing the visual discrimination task showed reduced left-right hemispheric asymmetries compared to those of the passively stimulated group in parts of the putamen and caudate nucleus. The most notable changes occurred within the body and head of the caudate, where left-right asymmetries virtually disappeared, due mainly to dramatic increases in right hemisphere activity presumably related to asymmetrical input from the somatosensory system. The body and head of caudate thus appear to serve multimodal functions in that visual activation of these loci in the left hemisphere was balanced by somatosensory activation of the same loci on the right. In the tail of the caudate, however, LCGU values remained higher on the left, indicating that this portion of the structure is strongly visual.

Computer-enhanced images of the autoradiographic brain sections from the animals in this experiment were examined in detail in order to delineate the exact borders of visually related tissue in the parietal and temporal lobes. We found that the cortical visual/nonvisual borders 1) were sharp and highly consistent among the animals, 2) outlined more visual tissue than expected in both the parietal and temporal lobes, and 3) appeared reliably at zones of architectonic transition.

Our data show that behind the junction of the lunate and the intraparietal sulcus all cortical tissue is related to vision. In front of this junction, nonvisual tissue first appears at the anterior or upper lip of the intraparietal sulcus. In the cross sections, one visual/nonvisual border can be placed on the medial surface, and one on the upper bank of the intraparietal sulcus. Anteriorly, both borders move in a ventral direction, with all tissue below them remaining visual. Still more anteriorly, this single expanse of visually related tissue is separated into two parts, parietal and temporal, by the appearance of nonvisual tissue in the lateral fissure and on the superior temporal gyrus.

In the parietal lobe, the upper border is always within the intraparietal sulcus, about halfway down the upper bank caudally and closer to the fundus rostrally. The lower border moves out of the lateral fissure and remains on the cortical surface close to the upper lip of the lateral fissure, and then it moves into the intraparietal sulcus rostrally. The rostral limit of visual tissue is within the intraparietal sulcus, about 5mm behind its anterior tip.

In the temporal lobe the upper border is always within the superior temporal sulcus, generally about halfway down the anterior (or dorsal) bank of the superior temporal sulcus but within the fundus rostrally. Anteriorly, the lower border moves from the calcarine fissure to the hippocampal sulcus (where it continues midway along its length) and then turns laterally to enter the occipitotemporal sulcus and finally the fundus of the rhinal sulcus.

These visual/nonvisual borders generally appear at zones of cytoarchitectonic transition described by von Bonin and Bailey. For example, in the parietal lobe a visual/nonvisual border appears on the lateral surface near the zone of transition between areas PG and PF and on the medial surface between prestriate area OA and parietal area PE. Also, in the temporal lobe, visual/nonvisual borders appear in the transition zones between TF and TH, TE and TH, and TE and TG. Finally, inside the expanse of visually related cortex, metabolic borders appeared to separate architecturally different subareas, as in the lower bank of the intraparietal sulcus and in the upper bank of the superior temporal sulcus. These results lend new functional validity to cortical architectonics.

Experiment 2

Portions of each of the visual areas within the cortical pathway serving object vision are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TEO and TE receive contralateral input via both the splenium and the anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we attempted to localize and to quantify the contribution to vision made by the commissural systems. To do this we measured LCGU throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy, and unilateral optic tract section alone. The 2-deoxyglucose method was applied one month postoperatively in awake rhesus monkeys actively viewing visual patterns. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of the two groups.

From the autoradiographs of each brain, representative sections were chosen at 1mm intervals throughout the extent of the cortical visual pathway. Weighted averages of LCGU for the entire extent of each visual area were then calculated from these sections by means of a computerized image-processing system. In the intact hemisphere there was a progressive decline in LCGU along the cortical visual pathway from a high of 66 μ moles/100g/min in area OC to a low of 47 in anterior TE. This sequential decline in the intact hemisphere was the same both for the animals with tract section plus commissurotomy and for those with tract section only. There were also no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere (ranging from 40% in OC to 60% in TEO). A difference attributable to visual input via the intact commissures was found in TE, however, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas, in animals with tract section only LCGU reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively. These results indicate that commissural inputs contribute more to the visual functions of area TE than to those of any other visual area and that, in fact, commissural inputs alone may be insufficient to support visual function in areas TEO, OA, and the OC-OB border.

Experiment 3

The results of Experiment 2 have presented us with a paradox. The rise in metabolic activity in the deprived hemisphere of the tract-cut preparations clearly reflect the functional contribution of the forebrain commissures in the anterior portion of the occipito-temporal pathway but, surprisingly, not in the posterior portion. Thus, on the one hand, our metabolic data on area TE are in good accord with existing anatomical data, which indicates that area TE receives interhemispheric projections through both the splenium and the anterior commissure. On the other hand, substantial commissural fiber projections also reach the more posterior visual areas - specifically, the OC-OB border, OA, and TEO. Yet our metabolic data give no indication of this heavy and widespread projection. A possible explanation of this paradox is that, unlike the commissural input to area TE, which clearly can support visual function by itself, the commissural input to the more posterior zone may be effective only against a background of spontaneous activity provided by an intact but visually occluded retino-geniculo-cortical pathway.

In order to test this hypothesis, we have prepared a series of monkeys in which a "blind" right hemisphere was produced by midline section of the optic chiasm combined with occlusion of the right eye rather than by right optic tract section. Through a comparison of LCGU in the right hemispheres of these animals and of those studied previously with right optic-tract section, the functional effectiveness of commissural input with and without spontaneous retinal input can be evaluated. Greater metabolic activity in the former case would indicate that the commissural fibers to the prestriate-posterior temporal zone do require a minimum level of background activity from the intact retina in order to make a functional contribution to vision.

Preliminary qualitative analysis, however, shows no reliable difference between animals with a tract cut and those with a chiasm cut and occluded eye either at the OC-OB border or within areas OB, OA, or TEO. In short, the commissural contribution to vision in the prestriate-posterior temporal zone appears not to be augmented by the presence of intact retinal fibers and spontaneous retinal activity. The functional difference between the commissural fibers serving the anterior and posterior portions of the occipito-temporal visual pathway thus requires some other explanation. Unit-recording studies are being planned to investigate the issue further.

Experiment 4

In order to trace the functional development of the visual system, we are conducting studies in infant monkeys similar to those described in the first experiment. Thus far, a series of animals with unilateral optic-tract section combined with forebrain commissurotomy have been tested at various ages ranging from two days to six months. As with the adults, representative brain sections were chosen at 1mm intervals throughout the entire cortical visual pathway in each animal, and weighted averages of LCGU for the entire extent of each visual area were calculated. Preliminary analysis shows that there are systematic age-related changes both in the absolute level of LCGU within the normal seeing hemisphere and in LCGU differences between the normal left and the deprived right hemisphere.

As in adults the normal hemisphere shows a gradient in which the absolute level of glucose utilization is highest in Area OC and is systematically reduced in the more anterior areas of the pathway, being lowest in the anterior portion of Area TE. This gradient was present even in the youngest subject, two days old. The steepness of this gradient, however, shows a consistent and nonoverlapping increase with age.

At all ages the deprived hemisphere shows reduced LCGU relative to the normal hemisphere. Also, at all ages, side-to-side differences are greatest in striate cortex and smallest in the anterior portion of the temporal lobe. However, the quantitative differences between the hemispheres change systematically with the age of the animal. Thus, for each cortical area, the relative difference between the right and left hemisphere is smallest at birth and approaches the difference seen in adults only at about four months.

These preliminary results agree with our behavioral data (see project report MH-00478) indicating that the neural capacity for visual recognition is probably not developed until about four months of age.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

Since energy metabolism and functional activity are highly correlated within the nervous system as demonstrated by these and other studies, the 2-deoxyglucose method can provide a deeper understanding than has been possible before of the role of various cerebral structures in behavior through both the identification of the structures involved in a particular behavior and the quantification of the degree of their participation. Since it is only through a knowledge of what is normal that we gain insight into what is abnormal, such understanding is certain to contribute greatly to the ultimate goal of diagnosing and treating a wide variety of neurological and mental disorders.

PROPOSED COURSE OF RESEARCH:

Our goal is to apply the 2-deoxyglucose method to the study of a variety of behavioral processes in the monkey, including perception, attention, memory, emotion, and volition for the purpose of identifying the various structures involved in these different behaviors and quantifying the degree of their participation. Our immediate plans are to investigate the neural structures involved in visual memory, specifically object recognition, a process which we believe involves not only the visual system but also parts of the limbic system (amygdala and hippocampus) and the medial thalamus.

In these experiments 2-deoxyglucose will be administered to monkeys performing a running recognition task designed to "load" the visual memory system throughout the experimental session. As in our previous studies, the monkeys will be prepared with a right optic tract section and section of the forebrain commissures to permit the flow of visual information through the left hemisphere only. By taxing visual memory we hope to see increased LCGU in

parts of the limbo-thalamic memory system (e.g., hippocampus and medial thalamic nuclei) in which they were not seen before.

We also plan to continue our investigation of the development of the visual system in infant monkeys, first completing the normative study under conditions of passive visual stimulation and then attempting to parcel out developmental differences between the "habit" and the "memory" systems (see project MH-00478).

PUBLICATIONS:

Macko, K.A., Jarvis, C.D., Kennedy, C., Miyaoka, M., Shinohara, M., Sokoloff, L., and Mishkin, M.: Mapping the primate visual system with 2-[¹⁴C]deoxyglucose. Science, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02034-04 LN |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Subcortical mechanisms related to frontal lobe functions in the monkey | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: H.E. Rosvold OTHER: D.P. Friedman R.K. Nakamura M. Mishkin M. Chieuh | Research Physiologist Senior Staff Fellow Senior Staff Fellow Act. Chief, Laboratory of Neuropsychology Staff Fellow | LN NIMH LN NIMH LPP NIMH LN NIMH LCS NIMH |
| COOPERATING UNITS (if any) Laboratory of Psychology and Psychopathology, NIMH | | |
| LAB/BRANCH Laboratory of Neuropsychology | | |
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| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 0.5 | OTHER: 0.5 |
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| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> The <u>dopamine</u>-containing neurons of the <u>substantia nigra</u> project to the <u>dorsolateral prefrontal cortex</u> and the <u>striatum</u>. In the <u>rheshus monkey</u>, electrolytic lesions of the prefrontal cortex or the related parts of the striatum, or depletion of dopamine in the prefrontal cortex, impair performance of <u>delayed alternation</u>, a test of <u>cognitive function</u>. However, bilateral destruction of the substantia nigra in a pilot animal had little, if any, effect. This project was undertaken to establish the generality of that negative finding. </p> | | |

PROJECT DESCRIPTION:

The purpose of this study is to examine the effects of lesions of the substantia nigra on delayed-alternation behavior and other cognitive functions in the monkey. This has become important because of conflicting data concerning the effects of nigral lesions on cognitive functioning.

Delayed alternation, a test of cognitive function, is severely impaired in monkeys by selective lesions of the dorsolateral prefrontal convexity, or of those portions of the striatum to which it projects. The dopamine (DA)-containing neurons of the substantia nigra send a powerful projection to the striatum. A similar, and fairly selective, projection to the dorsolateral prefrontal convexity has recently been demonstrated as well. Depletion of DA in this cortical region with the neurotoxin 6-hydroxydopamine impairs delayed-alternation performance in the monkey, and lesions of the DA-containing neurons of the substantia nigra and surrounding regions impair it in the rat. It therefore seemed likely that destruction of the substantia nigra in the monkey would also disrupt delayed-alternation behavior. Surprisingly, this has not turned out to be the case.

Lesions were aimed at the substantia nigra by combining stereotaxic and x-ray techniques to guide the approach in five animals. Each animal was retested on delayed alternation and visual discrimination (a control task). In addition, some of the animals were tested on a 24-hr. concurrent learning task to measure original acquisition of new information. A neurological test designed to detect deficits in motor and sensory function was also administered. Finally, two animals were challenged with haloperidol, a dopamine antagonist, and L-DOPA, a precursor known to increase dopamine levels, prior to retesting them on delayed alternation.

No convincing deficit was found in either delayed alternation, visual discrimination, or 24-hr concurrent learning. In initial neurological examinations following surgery, the animals appeared normal in motor ability, alert, and responsive to visual stimuli. There did appear to be some neglect of auditory and somatosensory input, but these deficits were neither severe nor long-lasting. Also, postsurgical injections of neither haloperidol nor L-DOPA affected delayed-alternation performance reliably.

Histological and biochemical analyses has now been completed in two of the animals. These indicate that the lesions were well placed, resulting in nearly total destruction of the substantia nigra; and they were also biochemically effective, yielding depletions of dopamine levels in prefrontal cortex and caudate nucleus of 90% and 80%, respectively.

The behavioral ineffectiveness of these lesions is puzzling, particularly in light of the severe behavioral changes that have been seen after substantia nigra lesions produced by injections of 6-hydroxydopamine (6-HD). The explanation for this discrepancy may be that the 6-HD injections damage behaviorally relevant structures in addition to those belonging to the

dopamine system. The alternative possibility that only total destruction of the dopamine system would yield any detectable impairment in performance seems less likely.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

Dopamine, a chemical transmitter produced particularly by neurons of the substantia nigra, has been heavily implicated in schizophrenia. The portion of the cerebral cortex that receives the heaviest input of dopamine is necessary for the performance of various cognitive tasks, among them the ability to perform delayed alternation. The nature of the dependence of the cortex on its dopamine inputs is as yet unknown, as is the precise relationship of dopamine to mental health and disease. It is the purpose of this project to provide such information, which eventually could be of both diagnostic and therapeutic use.

PROPOSED COURSE:

After completion of the remaining histological and biochemical analyses and preparation of a paper for publication, this project will be terminated in the next fiscal year due to the recent retirement of the PI.

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| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Anatomy of the primate visual system | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | |
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| PI: L.G. Ungerleider Senior Staff Fellow | | LN NIMH | | | | | | | | | |
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| R. Desimone | Staff Fellow | LN NIMH | | | | | | | | | |
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| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | |
| <p> The <u>sensory processing</u>, <u>perception</u>, and memory of visual events require the transmission of neural activity across a corticocortical, multisynaptic pathway from <u>striate cortex</u>, or <u>area 17</u>, through several <u>prestriate</u> "association areas". In addition, visual input from striate cortex reaches these prestriate areas indirectly via two subcortical visual structures, the <u>superior colliculus</u> and the <u>pulvinar</u>. By the combined use of <u>neuroanatomical techniques</u>, <u>electrophysiological recording</u>, and newly developed <u>histological staining</u> procedures, we have been identifying the multiple prestriate visual areas in the macaque, mapping their interconnections, and tracing the flow of visual information forward to the still higher-order visual areas located within the <u>temporal</u> and <u>parietal lobes</u>, visual areas that are critical for <u>object vision</u> and <u>spatial vision</u>, respectively. </p> | | | | | | | | | | | |

PROJECT DESCRIPTION:

Striate cortex, the primary visual cortex, is the source of two major corticocortical, multisynaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnects the striate, prestriate, and posterior parietal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, and inferior temporal areas, and is critical instead for object vision. Although visual information must reach the posterior parietal and inferior temporal cortex to enable their participation in spatial vision and object vision, respectively, the complex circuitry through which this information is transmitted has yet to be unraveled. We have undertaken to examine the details of the connections within these two cortical visual pathways, beginning with an analysis of the projections of the striate cortex itself.

Experiment 1:

Anatomical material from two series of monkeys (*Macaca mulatta*) was used to determine the locus, extent, and visuotopic organization of striate projections to prestriate cortex. One series was processed for terminal degeneration by the Fink-Heimer procedure following unilateral lesions of lateral, posterior, or medial striate cortex, areas representing central, peripheral, and far peripheral vision, respectively. Collectively, the lesions included all of area 17 with little or no invasion of area 18. The second series was processed for autoradiography following tritiated amino-acid injections into various striate sites representing the center of gaze and eccentricities ranging from 0.5° to 45° in either the upper or lower hemifield. The results indicate that striate cortex (cytoarchitectonic area OC), or V1, projects to at least three separate and topographically organized visual areas within prestriate cortex: V2, a circumstriate cortical belt; MT, located on the posterior bank and floor of the superior temporal sulcus; and V3a, located at the fundus of the posterior intraparietal sulcus. V2 corresponds to area OB, while MT and V3A are both contained within area OA.

Now that the total system of striate-prestriate projections has been delineated, the effects of completely disconnecting inferior temporal from striate cortex can finally be investigated. Accordingly, monkeys are being prepared with complete lesions of just the three striate projection fields described above (without inclusion of any striate tissue) prior to testing them on visual pattern discriminations. In addition, we are trying to determine whether even small prestriate lesions might disconnect inferior temporal cortex from corresponding parts of the visual field by testing discrimination of stimuli confined to those parts of the field in monkeys that have been trained to maintain fixation. In view of the clear visuotopic organization of the striate projection zones in both V2 and MT, even limited lesions within these prestriate zones should yield severe visual deficits provided the animals are forced to use the part of the visual field corresponding to the area damaged. To monitor fixation accurately, we are making use of a magnetic eye-coil system.

Experiment 2:

Having delineated the projections of striate to prestriate cortex, which we found to consist of three separate re-representations of the visual field, we are now following these projections further, with the inferior temporal and posterior parietal cortex as our targets. Since the multiple prestriate areas can be identified not only by their approximate location, but also by their distinct electrophysiological properties, all autoradiographic studies are now being performed under physiological control. By recording the activity of neurons from the microsyringe needle and mapping receptive fields, we are able to inject tritiated amino acids into portions of each prestriate area that represent known parts of the visual field. To date, this recording technique has been used for injections into both V2 and MT. In addition to tracing the projections of these two areas, we have developed a myeloarchitectural stain to distinguish among them and other prestriate regions.

Our results indicate that V2 projects to two visual areas located anterior to it, V3 and V4. Together, these three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like striate cortex, each belt contains a representation of the visual field, with the upper field located ventrally and the lower field, dorsally. Other studies have shown that V4, in turn, projects to both areas TEO and TE in the inferior temporal cortex. In contrast to V2, which appears to provide a major link forward from striate cortex into the temporal lobe, our results on MT suggest that it provides a major link from striate cortex into the parietal lobe via its projections to four additional areas in the superior temporal and intraparietal sulci. (These projection areas of MT, unlike those of V2, are highly convergent, with only a suggestion of topography.) It thus appears that there is a divergence in the flow of visual information from striate cortex which begins at the first prestriate area. Whereas information from V2 is mainly directed ventrally into the temporal lobe, information from MT is mainly directed dorsally into the parietal lobe, these two divergent projection systems providing the anatomical substrate for object vision and spatial vision, respectively.

Experiment 3:

Anatomical material prepared for Experiment 1 was used to investigate subcortical efferents from striate cortex to the pulvinar, a nucleus in the thalamus implicated in attentional mechanisms. We had found in Experiment 1 that striate projections to the prestriate cortical area V2 are visuotopically organized, and we had prior evidence of a similar topographic arrangement of pulvinar projections to V2. In the present study, we found that there is also a precise visuotopic organization of striate projections to the pulvinar, indicating the existence of two sources of striate input to V2 that are in perfect register: one, direct, i.e., corticocortical; and the other, indirect, via the pulvinar. This parallel system of inputs to V2 thus provides a possible mechanism by which activating signals (e.g., from dorsal cortical areas to the pulvinar via midbrain structures) acquire visual field specification, that is, a mechanism that tells the organism where in the field to attend. Although this hypothetical circuit may apply to all sensory

systems, for the visual modality our data indicate that only the inferior and lateral nuclei of the pulvinar are involved. Future anatomical studies will investigate the sources of midbrain input to the inferior and lateral pulvinar, as well as the organization of afferents to the pulvinar from other sensory modalities. In addition to the anterograde tracing techniques of degeneration and autoradiography, these studies will employ horseradish peroxidase for tracing retrograde axonal transport.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

An understanding of the basic mechanisms mediating normal vision is the first step in the prevention, diagnosis, and alleviation of sensory, perceptual, and mnemonic disorders. To this end, we have been exploring the projections of striate cortex, both to prestriate "association areas" and to subcortical visual structures. Our goal is to unravel the complex system of projections to the still higher-order visual areas located within the parietal and temporal cortex, areas critical for spatial vision and object vision, respectively. The combined use of axonal transport techniques and electrophysiological recording provides a powerful tool for tracing neural connections within these central visual pathways. In addition, the recent development of highly selective histological stains may give us the opportunity for the first time of identifying higher-order visual areas in the human brain that we have identified in the monkey.

PROPOSED COURSE OF RESEARCH:

To understand the role of visual association cortex in perception and memory we must identify the multiple functional areas that comprise this cortex, delineate their topographic organization, and explore the complex circuitry of their interconnections. So far, we have discovered that striate cortex is the source of two divergent cortical pathways, each with its own set of hierarchically organized prestriate association areas. We plan to study the further projections of these two pathways stepwise to the still higher-order visual areas located within the temporal and parietal lobes. A major question for the future will be how the object and spatial information carried in these two separate pathways are subsequently integrated anatomically to yield a unified visual percept. Ultimately, we will explore the links of both pathways to affective, memory, and motor systems by examining the projections of the multiple visual association areas to the limbic system, the prefrontal cortex, and the striatum.

PUBLICATIONS:

Ungerleider, L. G. and Mishkin, M.: Two cortical visual systems. In D. J. Ingle, M. A. Goodale and R. J. W. Mansfield (Eds.) Analysis of Visual Behavior, The MIT Press, Cambridge, MA., 1982, pp. 549-586.

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|---|--|---|-------------------------------|-------------|--------------|---------|--------|---------------|-----------------|-------------------------------|--|------------|-----------|-----------------|--|------------|---|---------|--|-------------|--------|--------|--|-------------------|-------------------------------------|--------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02036-02 LN | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Neural coding of visual stimuli in the immobilized monkey | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 40%;">R. Desimone</td> <td style="width: 30%;">Staff Fellow</td> <td style="width: 10%;">LN NIMH</td> </tr> <tr> <td>OTHER:</td> <td>E.L. Schwartz</td> <td>Asst. Professor</td> <td>New York Univ. Medical School</td> </tr> <tr> <td></td> <td>C.G. Gross</td> <td>Professor</td> <td>Princeton Univ.</td> </tr> <tr> <td></td> <td>M. Mishkin</td> <td>Act. Chief, Laboratory of Neuropsychology</td> <td>LN NIMH</td> </tr> <tr> <td></td> <td>S.J. Schein</td> <td>Expert</td> <td>CB NEI</td> </tr> <tr> <td></td> <td>F.M. DeMonasterio</td> <td>Chief, Section on Visual Processing</td> <td>CB NEI</td> </tr> </table> | | | PI: | R. Desimone | Staff Fellow | LN NIMH | OTHER: | E.L. Schwartz | Asst. Professor | New York Univ. Medical School | | C.G. Gross | Professor | Princeton Univ. | | M. Mishkin | Act. Chief, Laboratory of Neuropsychology | LN NIMH | | S.J. Schein | Expert | CB NEI | | F.M. DeMonasterio | Chief, Section on Visual Processing | CB NEI |
| PI: | R. Desimone | Staff Fellow | LN NIMH | | | | | | | | | | | | | | | | | | | | | | | |
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| | C.G. Gross | Professor | Princeton Univ. | | | | | | | | | | | | | | | | | | | | | | | |
| | M. Mishkin | Act. Chief, Laboratory of Neuropsychology | LN NIMH | | | | | | | | | | | | | | | | | | | | | | | |
| | S.J. Schein | Expert | CB NEI | | | | | | | | | | | | | | | | | | | | | | | |
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| COOPERATING UNITS (if any) Princeton University, New York University Medical School | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neuropsychology | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <p> The neural mechanisms for the <u>visual recognition</u> of objects extend beyond striate cortex into the surrounding prestriate and inferior temporal areas. Neurons in the prestriate areas appear to process local properties of objects such as the location of boundaries, while inferior temporal neurons appear to process more global features such as object shape. We are studying <u>single neurons</u> in prestriate and inferior temporal cortex to investigate both mechanisms. In <u>prestriate cortex</u> we have found that one area, area MT, is specialized for analyzing <u>stimulus motion</u> and contains <u>direction-of-motion columns</u> similar to the orientation columns discovered in primary visual cortex. In another area, <u>area V4</u>, we have found that neurons are sensitive to the <u>length, width, and color</u> of object contours and may play a role in separating figure from ground. In <u>inferior temporal cortex</u> we found that over half the neurons were tuned to a set of <u>shape descriptors</u> that can be used to code object shape. Since different neurons are tuned to different descriptors, a population of inferior temporal neurons could code any shape. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | |

PROJECT DESCRIPTION:

Previous work in this and other laboratories has shown that the neural mechanisms for the recognition of objects extend beyond the primary visual cortex into the surrounding prestriate and inferior temporal visual areas. Neurons in the prestriate areas receive their visual input directly or indirectly from striate cortex, have small receptive fields, and appear to process the local features of objects, such as individual contours and surfaces. Neurons in inferior temporal cortex receive their visual input from prestriate cortex, have large receptive fields, and appear to process the global features of objects, such as their shape. In this project we are studying both types of processing, local and global, in prestriate and inferior temporal cortex. To study the passive visual properties of neurons, unaffected by eye movements or the changing state of the animal, we are recording neural activity in the immobilized, lightly anesthetized macaque. We thus have complete control over the pattern of stimulation on the retina, and we are able to study individual neurons in this way for many hours.

Experiment 1: Direction-of-motion columns in prestriate cortex:

The concept of columnar organization, i.e., the subdivision of the major structures of the brain into columns or modules of neurons united by a common task, is of fundamental importance in understanding the organization of the nervous system. In the visual system the only columnar systems discovered so far have been in the primary visual cortex. As described by Hubel and Wiesel, the primary visual cortex contains elaborate columnar systems for analyzing stimulus orientation and ocular dominance. If we could find columnar organization for other stimulus dimensions within the prestriate visual areas, this would provide us with the best evidence yet of the functions of these areas. We chose the prestriate area 'MT' to look for such an organization because the location, borders, topographic organization, and anatomical connections of MT have already been well established by other experiments in the laboratory.

We discovered that area MT contains a columnar architecture for analyzing the direction of stimulus motion. In three monkeys, we recorded from 614 single neurons on 21 electrode penetrations. The majority of cells respond to a single direction of stimulus motion within their receptive field. In a vertical column of cells, all cells have the same receptive field and respond to the same direction of motion. Moving horizontally within the cortex, the optimal direction of motion changes smoothly and systematically from column to column. The representation of direction of motion in MT is strikingly similar to the representation of orientation in striate cortex. Even the size of the columnar systems is similar - 180 degrees of direction of motion in MT is represented within a piece of cortex 400 to 500 microns wide, the same size as the representation of 180 degrees of stimulus orientation in striate cortex. These results suggest that just as the analysis of stimulus orientation is a fundamental function of striate cortex, the analysis of stimulus direction of motion is a fundamental function of area MT. Anatomical experiments in the laboratory indicate that MT sends this information primarily to the spatial system of the parietal lobe but also, through intermediate projections, to the pattern system of the temporal lobe.

Experiment 2: Analysis of form and color in prestriate cortex:

While MT neurons are sensitive to stimulus motion, we have found neurons in another prestriate area, area V4, that are sensitive to the form and color of stationary stimuli. Although our experiments are still in progress, it is already clear that V4 neurons are sensitive to many different local features of objects, including the length, width, orientation, color, contrast, and spatial frequency of individual object contours. Unlike most neurons in striate cortex, the receptive fields of V4 neurons are surrounded by large suppressive regions. Thus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or contrast. These neurons may thus play a role in separating 'figure' from 'ground', a fundamental task in visual perception. Anatomical experiments in the laboratory have found that V4 provides a crucial link in the relay of visual pattern information into the inferior temporal cortex.

Experiment 3: Shape recognition and inferior temporal cortex:

A likely site for mechanisms of shape recognition is inferior temporal (IT) cortex. In man and monkey, removal of this area impairs visual recognition of shapes and patterns while leaving basic visuosensory capabilities intact. Furthermore, unlike neurons in prestriate cortex, many IT neurons are sensitive to the overall shape of objects rather than the location and quality of individual edges and contours.

In this study we examined how IT cortex might extract information about the overall shape of an object from information about its boundary. We adopted a method of representing shapes in terms of local boundary orientation that is used in computer pattern-recognition systems. The method depends on extracting a set of periodic features, known as the Fourier Descriptors, from the boundary of the object. Any shape is fully described by its set of Fourier Descriptors, or FDs, and a smaller set of only the low-frequency terms can often provide the 'gestalt' of a shape. Furthermore, this method of describing shape is independent of both the position and size of the stimulus. Thus, the FDs are a powerful and efficient alphabet for representing and classifying shapes.

Could IT neurons code shape on the basis of global shape features like the Fourier Descriptors? To explore this possibility, we created a set of stimuli from single FDs. If IT neurons function as 'bandpass filters' for shape, one would expect different IT neurons to be tuned to different FD stimuli, and the tuning should be relatively independent of the size and position of the shape on the retina. The activity of a set of such neurons could specify or code any complex shape.

We studied 234 neurons in five monkeys. About half of the neurons were tuned to different FD stimuli. For two-thirds of the tuned cells, the shape of the tuning curve remained invariant over changes in the size of the stimulus and its position on the retina. These results support the possibility that the visual system, and inferior temporal cortex in particular, use periodic shape

descriptors in classifying objects. We now plan to test whether the responses of neurons to FD stimuli can be used to predict their response to complex objects.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

The primate, including man, is a highly visual animal. Thus, it is not surprising that perhaps half of the primate cerebral cortex is devoted directly or indirectly to visual processing. Consequently, the study of neural mechanisms of vision has proven to be not only of fundamental importance for our understanding of visual perception and memory but also for our understanding of brain function in general. The extrastriate visual areas described in this project are of particular importance to the field of neurobehavioral research because they contain the neural mechanisms for visual memory, and they are the direct source of nearly all the visual information to the limbic affective and motivational systems.

PROPOSED COURSE OF RESEARCH:

Our findings in the past year that neurons in one prestriate area are organized within direction-of-motion columns, that neurons in a different prestriate area code contour and color, and that neurons in inferior temporal cortex appear to code object shape all indicate that the study of single neurons can give us valuable insight into the neural mechanisms of perception and memory. Clearly we have only scratched the surface. We hope to follow the flow of motion information into the parietal visuospatial system and study how parietal neurons use that information to code the spatial relations among objects. In addition, within the system for pattern vision, we plan to study how prestriate neurons use information about contours and colors to separate figure from ground and how inferior temporal neurons integrate information about object shape with other object features, such as texture, depth, and color.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 02037-01 LN | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Functional anatomy of the somatosensory cortex of the monkey | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: D.P. Friedman</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LN NIMH</td> </tr> <tr> <td>OTHER: E.A. Murray</td> <td>Staff Fellow</td> <td>LN NIMH</td> </tr> <tr> <td>M. Mishkin</td> <td>Act. Chief, Laboratory of Neuropsychology</td> <td>LN NIMH</td> </tr> <tr> <td>R.J. Schneider</td> <td>Guest Worker</td> <td>LN NIMH</td> </tr> </table> | | | PI: D.P. Friedman | Senior Staff Fellow | LN NIMH | OTHER: E.A. Murray | Staff Fellow | LN NIMH | M. Mishkin | Act. Chief, Laboratory of Neuropsychology | LN NIMH | R.J. Schneider | Guest Worker | LN NIMH |
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| COOPERATING UNITS (if any) | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The pathway by which somatosensory information reaches the limbic structures in the temporal lobe known to be critical for tactile memory has not yet been identified. To trace this pathway, the anatomical connections of identified <u>somatosensory fields</u> lying in or near the lateral sulcus of the <u>macaque monkey</u> have been investigated using both <u>anterograde</u> and <u>retrograde axonal transport techniques</u>. The data show that a series of <u>parallel tactile processing pathways</u> converge on the <u>insular cortex</u>; this region, in turn, projects directly to the amygdala and indirectly to the hippocampus via the rhinal cortex, thus linking the <u>first (SI) and second (SII) somatosensory cortices</u> with the <u>limbic structures</u> of the temporal lobe. The <u>laminar pattern of termination</u> of these projections suggests a sequential order in which the somatosensory fields process information. There appears to be a "forward" sequence of projections (with terminations mainly in layer IV of the cortex) proceeding from SI through the other somatic fields to the insular cortex and from there to the limbic system; these connections may serve as a hierarchically organized cortico-limbic pathway for <u>tactile perception</u> and <u>memory</u>. </p> | | | | | | | | | | | | | | |

PROJECT DESCRIPTION:

Objectives:

Work in this laboratory has shown that the amygdala and hippocampus are critical not only for visual memory but also for tactual memory. Though these studies suggested that the second somatosensory area (SII) and the insular cortex may act as relays for the transfer of somatic inputs from the first somatosensory area (SI) to the limbic system, it is known that, in addition to SII, a number of other fields in or near the lateral sulcus also receive somatic inputs. Because little is known about the connectivity or other properties of these fields, we have undertaken to study them with the goal of delineating (I) the route via which somatosensory information reaches the limbic structures critical for memory, (II) the corticocortical interrelations of the somatic fields involved, (III) the thalamic relationships of these fields, and (IV) their single-unit response properties.

Methods employed:

Both anterograde (autoradiographic) and retrograde (horseradish peroxidase [HRP]) transport techniques were employed to examine the neuroanatomical connections of the cortical fields of interest. Single and multi-unit recording techniques were used to identify the specific cortical fields in the lateral sulcus of the macaque that are activated by somatic input. These fields include the second somatosensory (SII) cortex, area 7b, the retroinsular cortex, and the granular and dysgranular insular fields. After a particular field was mapped, an injection of either tritiated amino acids (a mixture of proline and leucine) or HRP was made into the hand or digital representation within it to trace its connections. Accurate placement of the injection was ensured by either i) injecting through the recording pipette by iontophoresis or ii) recording from a microelectrode cemented to the needle of the injection syringe.

Histologic identification of cortical fields has been improved through processing of adjacent sections to reveal either cell bodies, with a standard Nissl stain, or axons, with a sensitive silver stain we have developed for bulk use.

Physiological studies of lateral sulcus neurons have been performed both in immobilized monkeys, which were lightly anesthetized, and in awake monkeys restrained in a primate chair.

Major findings:

I. Corticocortical Connectivity:

Using the combined recording-injection techniques described above, we have placed injections into SII, area 7b, area 5, the retroinsular field (Ri), and the granular (Ig), dysgranular (Id), and agranular (Ia) insular fields. By combining the data concerning anterograde projections derived from the

tritiated amino acid injections and retrograde projections derived from the HRP injections we have demonstrated reciprocal connections between: SII and Ri, SII and area 7b, SII and Ig and Id, and Ri and Ig. Also, we have confirmed previously reported reciprocal projections between SI and SII and demonstrated reciprocal projections between area 5 and both Ri and area 7b. Finally, anterograde labeling resulting from HRP injections into the insular fields has confirmed recently reported projections from Ig and Id to the amygdala and from Id to the prorhinal and perirhinal cortical areas. These areas, in turn, send major inputs to the hippocampus.

Our studies thus demonstrate that tactual information may reach the amygdala and hippocampus via relays in the granular and dysgranular insular fields, which receive their somatic cortical inputs from SII and Ri. A ventrally directed cortico-limbic pathway originating in SI may therefore be important for the perception and memory of somatosensory stimuli. This possibility is now being examined in a series of ablation studies.

II. Laminar Patterns of Termination:

Three different laminar patterns of terminal fields of the corticocortical projections described above were seen. Each pattern depended on the field into which the injection was made and the field to which the injected field projected. Though similar patterns have been described in other areas of the cortex, only one has previously been reported in the somatosensory system.

This pattern consists of a heavy band of labeled terminals in layer IV, with progressively lighter labeling, indicative of fewer terminals, in the supragranular layers, III, II, and I. There is a light band of terminal labeling in layer VI paralleling that seen in layer IV. This pattern has been described for the projections from SI to SII and area 5, and from area 5 to Ri and 7b. It is similar to the forward (i.e. outward from striate cortex) projections seen in the visual system.

The second pattern is analogous to what has been described in the visual system as a backward projection (i.e. towards the striate cortex). Its most striking characteristics are a complete absence of labeling in layer IV and heavy labeling in layer I. Additional labeling is seen in layer VI and sometimes in layers III and II. The projections from SII to SI and Ri, from Ri to area 5, and from Ig to SII and Ri are all of this type.

The third pattern, previously described in prefrontal association areas, consists of a single, apparently homogeneous column of labeled terminals extending from layer VI through layer I. Its most striking feature is the lack of laminar differences in labeling density, in sharp contrast to the so-called forward and backward projections described above. This pattern is seen in the projection from SII to area 7b.

By analysing the pattern of forward and backward projections we have been able to determine the probable sequential order in which information is processed in the somatosensory system. The forward direction is SI to SII and area 5,

area 5 to Ri and area 7b, area 7b and Ri to SII, and SII and Ri to Ig. SII also projects to Id. Ig and Id then project to the limbic system.

III. Thalamocortical relations:

We have begun to reexamine the thalamic connectivity of the cortical somatic fields because our preliminary findings suggest that the thalamic relations of these fields differ in many respects from those reported in the literature, which is sparse. By having, for the first time, an appreciation of the full extent of these fields, and by using our combined recording-injection techniques to increase the accuracy of our injections, we should be able to provide the best account yet of this important anatomical relationship. Only previously unreported findings will be described here.

We have seen widespread projections from the medial pulvinar (Pulm) to a number of somatic or polysensory fields, including previously unreported inputs to Ig, Id, and area 7b. Since Pulm is known to project to the polysensory area in the superior temporal sulcus and to the amygdala, these new findings raise interesting questions about the role of this thalamic nucleus in learning and memory.

Our injections in SII have consistently resulted in heavy labeling in the ventroposterior inferior (VPI) nucleus as well as in the most ventral parts of the ventroposterior lateral (VPL) nucleus. Because SII is widely believed to receive its thalamic input exclusively from VPL, this finding could dramatically alter the current view of the organization of the somatosensory system.

Similarly, it is currently held that VPI is a relay to Id, yet our injections of HRP into Id have never labeled neurons in VPI, whereas they have labeled neurons in the ventroposterior medial nucleus, pars parvocellularis (VPMpc), immediately medial to VPI. This nucleus is considered to be a thalamic relay for taste rather than somatic information. Like VPI, VPMpc has not been extensively studied, and our findings relating it to the somatic system suggest that a major reevaluation of this portion of the thalamus needs to be undertaken. We have also seen previously unreported projections from the thalamic midline nuclei to Id.

IV. Physiological Studies of Insular Cortical Neurons:

This project has just been initiated. Preliminary results indicate that Ig and Id are higher order somatic processing areas because neurons there, like those in higher order visual areas, have large, bilateral receptive fields that can be activated by inputs from a number of submodalities. Our initial studies will be directed at describing the types of input most effective in activating these units and in delineating the areas of the insula so activated.

SIGNIFICANCE TO BIOMEDICAL AND BEHAVIORAL RESEARCH:

Studies concerning the connections of the somatosensory system have been relatively restricted in scope. This project supplies the first comprehensive look at the entire somatosensory system and how it may connect with the limbic structures necessary for memory. Furthermore, this project is yielding fundamental insights into how the cerebral cortex processes information by describing the precise laminar pattern of connections of each pair of fields and by adding new data about the thalamic connections of these fields. As a whole, our studies have demonstrated remarkable parallels between the organization of the somatosensory and the visual systems, suggesting that common mechanisms of perception and memory operate within both, and that further studies of each one will illuminate the other.

PROPOSED COURSE OF RESEARCH:

In order to understand the processing of somatosensory information, we must know which cortical fields are involved, the interconnections of these fields, how they respond to specified inputs, and how such responses are modified by other regions of the brain. We now have, for the first time, considerable data concerning the extent and connectivity of the cortical fields of the somatosensory system. However, we still know little about their topographic relations and about their connections with the thalamus and other subcortical structures. One goal for the future will be to fill in these missing data by continuing with the types of anatomical experiments described in this report. Also, virtually nothing is known about the physiology of the insular cortical neurons that receive somatic input, and so a second goal will be to study their response properties in a systematic manner in awake monkeys.

The third goal, to which we expect to make a major commitment, is to assess the influences of other brain regions, especially the limbic system, on information processing in general. As a first step, we will look at the distribution of known transmitter substances and neuropeptides, especially the endogenous opiates, within those areas of cortex we know to be important in learning and memory and that we already know have high levels of opiate receptors. Once we obtain these data, we hope to explore the effects of specific neuropeptides on learning and memory when their levels are altered within specific regions of the limbic system and neocortex.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00471-27 LPP | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Studies of Heredity and Environment in Schizophrenia | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">David Rosenthal</td> <td style="width: 20%;">Guest Worker</td> <td style="width: 25%;">LPP NIMH</td> </tr> <tr> <td rowspan="4">OTHERS:</td> <td>Allan F. Mirsky</td> <td>Chief</td> <td>LPP NIMH</td> </tr> <tr> <td>Edward K. Silberman</td> <td>Clinical Associate</td> <td>LPP NIMH</td> </tr> <tr> <td>Shmuel Nagler</td> <td>Research Psychologist</td> <td>Institute for Research on Kibbutz Education (Israel)</td> </tr> <tr> <td>Olive W. Quinn</td> <td>Guest Worker</td> <td>LPP NIMH</td> </tr> <tr> <td></td> <td>Patricia Lowing</td> <td>Staff Psychologist</td> <td>William Beaumont Hospital</td> </tr> <tr> <td></td> <td>Arje Latz</td> <td>Associate Professor</td> <td>Boston University</td> </tr> </table> | | | PI: | David Rosenthal | Guest Worker | LPP NIMH | OTHERS: | Allan F. Mirsky | Chief | LPP NIMH | Edward K. Silberman | Clinical Associate | LPP NIMH | Shmuel Nagler | Research Psychologist | Institute for Research on Kibbutz Education (Israel) | Olive W. Quinn | Guest Worker | LPP NIMH | | Patricia Lowing | Staff Psychologist | William Beaumont Hospital | | Arje Latz | Associate Professor | Boston University |
| PI: | David Rosenthal | Guest Worker | LPP NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| OTHERS: | Allan F. Mirsky | Chief | LPP NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| | Edward K. Silberman | Clinical Associate | LPP NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| | Shmuel Nagler | Research Psychologist | Institute for Research on Kibbutz Education (Israel) | | | | | | | | | | | | | | | | | | | | | | | | |
| | Olive W. Quinn | Guest Worker | LPP NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| | Patricia Lowing | Staff Psychologist | William Beaumont Hospital | | | | | | | | | | | | | | | | | | | | | | | | |
| | Arje Latz | Associate Professor | Boston University | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Harvard University; Institute for Research on Kibbutz Education; William Beaumont Hospital, Michigan; and Boston University | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUT AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">4.0</td> <td style="text-align: center;">3.0</td> <td style="text-align: center;">1.0</td> </tr> </table> | | | TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | 4.0 | 3.0 | 1.0 | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4.0 | 3.0 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSULS <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The project is composed of the following studies: (1) An intensive multi- disciplinary study of a family with MZ <u>quadruplets</u> (daughters) concordant as to <u>schizophrenia</u> but discordant as to severity and outcome. (2) Studies of <u>adoptees</u> and their <u>biological</u> and <u>adoptive</u> families. (3) A study of children (of schizo- phrenic and control parents) reared in town or <u>kibbutz</u> in Israel. | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. We are continuing our contacts with this family to see what happens in the clinical course of these women and to see how the course is related to earlier and to current life experiences; (2) Studies of adoptees and their biological and adoptive families in Denmark; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

(1) The Genain Quadruplets.

In 1963 David Rosenthal published the results of an extensive study of a group of four women, identical quadruplets, all of whom had succumbed to schizophrenic illness at some point during their late teens or early twenties. The women (who were named by Rosenthal for this publication: Nora, Iris, Myra, and Hester, i.e., N.I.M.H.) were studied by a group of psychologists and psychiatrists at the NIMH and were examined with virtually all of the methods extant in the late 1950's for studying schizophrenia and psychological deficit. After a period of study at the NIMH which extended over several years and which relied heavily on psychiatric treatment of the dynamic variety (both as therapy and as a means of gaining information), Rosenthal summarized the investigative effort in the Genains by the suggestion that the diathesis-stress theory was a reasonable way of accounting for the differences in the severity of their psychiatric illness. Although they shared an identical heredity (diathesis), differences in the way they were treated by their parents and significant others in their environment led to different expectations and self-pictures and consequently to different phenotypic expression of the schizophrenic disease. The more competent "pair", Nora and Myra, were more favored and fussed over; the smallest and least prepossessing physically, Hester, was most often bracketed with Iris. Willy-nilly, Hester and Iris were treated as the less competent and capable pair and more or less fulfilled that expectation. This is a somewhat oversimplified but reasonably accurate summary of the earlier view of the Genains.

Rosenthal and his early colleague and collaborator, Olive Quinn, maintained contact with the Genains and with their mother (who at 83 still watches over her brood). Through Rosenthal's and Quinn's good offices and contacts, we were able to persuade the Genain clan to return to NIMH for another period of study. In addition to the quadruplets themselves, the group included the mother, the husband of Myra (she is the only one to have married) and Myra's two adolescent sons. On this occasion, which lasted for a period of approximately 3 1/2 months, we tested the Genains with the full battery of neurobiological test procedures that have evolved over the last 25 years. The procedures included: an extensive series of genetic identity tests; biochemical determinations from blood, urine, and cerebrospinal fluid of various catecholamine compounds with emphasis on dopamine and norepinephrine; procedures related to the identification of possible preexisting viral infection of the central nervous system; neuroradiological and

neurophysiological tests (CT scan, PETT scan, evoked potential and EEG brain maps, brain stem evoked potentials); and an exhaustive battery of psychological and psychometric tests with a special focus on measurement of attention, arousal and memory. Two of the tests were essentially identical to measures employed in the late 50's--the continuous performance test and the reaction time paradigm. Further, for most of the behavioral tasks, we were able to examine the Genains both on and off medication--the latter after a period of at least two weeks free from the phenothiazine drugs they were taking on admission to the NIMH.

We conclude that the Genains are functioning about as well as they ever have in their adult lives, and scores on attention tests show improvement as compared with 1958 measures. This is likely attributable to the medication (primarily phenothiazines) and other supportive treatments they have received over the years. With respect to the varying degrees of illness seen in the Genains, the following findings appear relevant: the tests indicate that two of the women (Nora and Hester) deteriorated rapidly when removed from medication, and two (Iris and Myra) did not. The consequence of this is that the grouping of the quadruplets on the basis of their characteristics and abilities while they are medicated is different from that apparent while they are off medication. On medication the apparent pairing is Nora and Myra (as before) and Hester and Iris. Scrutiny of the test material, including the biochemical, physiological, neuroradiological and immunogenetic, as well as behavioral, leads to speculation that certain unique biochemical findings and differing types and amounts of cerebral pathology may constitute the fundamental cause of the variable expression of schizophrenia in the Genains. This set of circumstances is superimposed on a basic schizophrenic diathesis which is manifest in the biochemical and certain neurological and neurobehavioral findings. The interdisciplinary research effort represented by this series of studies is unique in the annals of schizophrenia research and has led, we believe, to testable hypotheses on the role of various neurobiological factors in the development, etiology, and expression of the disease.

The studies are currently being prepared for publication as a series of three papers to appear in Psychiatry Research.

(2) The Danish Adoptee Study--Reanalysis of the Data.

Using data from Danish health records, in a now-classic study, Rosenthal, Kety and Wender compared the frequency of schizophrenia spectrum disorders in two groups of persons adopted in infancy or early childhood: those with a psychotic parent (index group) and those whose biological parents had never had psychiatric treatment (control group). Significantly more disorder was found in the index than the control group. This study has been criticized recently on the grounds that subjects were included inappropriately (affective rather than schizophrenic diagnoses in the parents; insufficient information available about the father). We have completed a reanalysis of the original material using the new DSM III methods, and stricter exclusionary criteria applied to the parents. The results of the reanalysis yielded three times as many schizophrenia spectrum disorders in the index as in the control group, a slightly better result than that found in the original Rosenthal et al., study. The difference between groups remains statistically significant, supporting the operation of genetic factors in the

transmission of schizophrenia spectrum disorders. The manuscript describing the findings has been submitted to the American Journal of Psychiatry for review and possible publication.

(3) The Israel Kibbutz--High Risk Study.

During the past year, the Laboratory has been engaged in completing work on the study of children at risk for schizophrenia in Israel, which was designed and initiated by Dr. David Rosenthal. The study has examined 100 children, of whom 50 had one schizophrenic parent, and 50 were born to two nonschizophrenic parents. Half of both "index" and control groups were reared in towns in traditional nuclear families, while the remaining half were reared in communal settings on kibbutsim.

Our work has been in two phases. The first has been to complete data analysis of the initial examination of subjects, done when they averaged 11 years of age, and prepare manuscripts for the first major publication of the results. At the present time, data analysis is complete, and manuscripts are in the final stages of completion (for publication in the Schizophrenia Bulletin). In broad outline, the results indicate that index children were discriminable from controls in many areas of function, but kibbutz and town children did not differ on the experimental examinations. Furthermore, kibbutz versus town rearing had no discernible effect on the performance or behavior of high-risk children. Index children were found to be poorer in psychosocial adjustment, perform more poorly in school, manifest a number of neurological "soft signs", and show deficits on psychological tests requiring high levels of attention, visual integration, and visuo-motor coordination. An important negative finding was lack of differences between index and control children on psychophysiological measures of arousal and habituation.

The second phase of the study has involved the collaboration of Dr. Arje Latz, of Boston University, who has been engaged in conducting follow-up interviews with study subjects. These subjects are now in their mid-twenties, at the peak of their risk period for schizophrenic breakdown. Ninety of the original subjects have been seen at this writing, and arrangements are being made to contact a number of others who are no longer in Israel. Preliminary results show that nine subjects fall within the "schizophrenia spectrum" (of whom six are DSM III schizophrenic), six from kibbutz backgrounds, and three from towns. When all DSM disorders are considered, more than five times as many ill subjects fall within the index as within the control group. Furthermore, when schizophrenia itself is excluded, the remaining subjects with history of illness (including DSM III Major Affective Disorder or Dysthymic Disorder) are found predominantly in the index-kibbutz cell. Other significant preliminary results include persistence of attention-related deficits in the index group, and continued poor social and work adjustment in high-risk subjects.

At present, work on the project centers around completion of data collection for the adult follow-up study. These data will then be compared to information from the previous cross-sectional studies with this group in an attempt to elucidate possible precursors of adult illness.

Significance to biomedical research and to the program of the Institute:

The issue of the mode of heritability of mental illness, and factors which modify it, may be the highest priority of the Institute. This work contributes significantly to our knowledge in this area and ultimately, to our capacity to treat and prevent schizophrenia and related disorders.

Proposed course:

The remaining data analyses will be completed and the work will be prepared for publication. No new data gathering is planned at this time. It is estimated that two years will be necessary for completion of this work.

Publications:

Rosenthal, D.: The Genetic Environmental Perspective in Psychopathology. In Al-Issa, I. (Ed.): Culture and Psychopathology. Baltimore, University Park Press, 1982, pp. 111-122.

Rosenthal, D., Nagler, S. et al. : The Israeli High Risk Study. Schizophrenia Bulletin, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00472-19 LPP | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Investigations of Some Formal Characteristics of Speech | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: Theodore P. Zahn</td> <td style="width: 33%;">Research Psychologist</td> <td style="width: 33%;">LPP NIMH</td> </tr> <tr> <td>OTHER: Donald S. Boomer</td> <td>Guest Worker</td> <td>LPP NIMH</td> </tr> </table> | | | PI: Theodore P. Zahn | Research Psychologist | LPP NIMH | OTHER: Donald S. Boomer | Guest Worker | LPP NIMH |
| PI: Theodore P. Zahn | Research Psychologist | LPP NIMH | | | | | | |
| OTHER: Donald S. Boomer | Guest Worker | LPP NIMH | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL MANYEARS: 0.0 | PROFESSIONAL: 0.0 | OTHER: 0.0 | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This research project investigates <u>attention</u> during natural <u>speech perception</u> . Specifically, we are attempting to test the hypothesis that speech perception involves phasic, rather than continuous attention, and is characterized by bursts of cognitive activity at linguistically specified boundaries in the stream of speech. The method involves the measurement of <u>reaction time</u> to irrelevant stimuli--clicks--while the subject is listening to a tape-recorded dialogue. If the hypothesis is correct, the responses should be relatively inhibited for those clicks timed to occur during the postulated bursts of cognitive activity at linguistic boundaries. If the fundamental hypothesis can be given empirical support, this method will be used to investigate <u>attention deficit in schizophrenia</u> . This project has been temporarily inactive. | | | | | | | | |

Project Description

The guiding hypothesis of this study, growing out of work on listener responses is that listeners, like speakers, process speech in "chunks," or temporal patterns, the chunks being phonemic clauses. If the decoding process operates with phonemic clause speech units, then attention to the incoming speech need not be continuous. We hypothesized that attention would be maximally occupied at the end of the phonemic clause and minimally occupied while the clause is being accumulated and stored.

In the present study, subjects listen to a rather absorbing tape-recorded dialogue in one ear while also attending to an irregular series of clicks presented to the other ear. They respond to each click by depressing a telegraph key as quickly as possible. Reaction times are measured and stored. Half of the clicks are precisely timed to occur at the end, or terminal juncture, of phonemic clauses. Our hypothesis is that the subjects' attention to the cognitive demands of speech decoding will be different during clauses than at the ends of completed clauses, and this difference will be reflected in the mean response latency to the two sets of linguistically-specified clicks.

We found that for right-handed subjects, when speech is presented in the right ear and clicks in the left, reaction times were about 20 msec. faster to clicks at the terminal juncture than to those during the clause (18 of 20 subjects showed this effect). The data suggest that fluctuations of attention do occur during speech perception, but contrary to the original hypothesis, speech processing seems to be more active during a clause than at the terminal juncture. We have tested 20 right-handed subjects, with speech going to the left ear and the clicks to the right ear, and have tested a number of left-handed subjects on the original speech--right ear mode. Most of these subjects also show faster reaction time at the terminal juncture but to a somewhat lesser degree than the original group. However, left-handed males seem not to show any clear effect of click placement.

Significance to biomedical research and to the program of the Institute:

Beyond the present normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular attention to the specification of hemispheric asymmetry in cortical function. It is widely believed that cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically the ability to extract the salient elements of complex stimulus patterns. Since speech is just such a complex pattern and since the hemispheres are asymmetrically specialized for speech, a psycholinguistic approach to the problem of laterality and attention seems to be both neglected and promising.

Proposed course:

We will try to recruit more left-handed males to make a total of 20 subjects in that group. Future plans depend on the statistical analysis of the data.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00484-22 LPP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Psychophysiological Responsitivity and Behavior in Schizophrenia</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Theodore P. Zahn OTHER: Carmi Schooler Monte Buchsbaum Dennis Murphy Daniel VanKammen Thomas Robinson, Jr. Suzanne Haynes Larry Siever Thomas Insel | Research Psychologist Research Psychologist Research Psychiatrist Chief Research Psychiatrist Guest Worker Epidemiologist Clinical Associate Clinical Associate | LPP NIMH LSES NIMH BPB NIMH CNB NIMH BPB NIMH LPP NIMH HV EP NHLBI CNB NIMH CNB NIMH |
| COOPERATING UNITS (if any) Laboratory of Socioenvironmental Studies, Biological Psychiatry Branch, Laboratory of Clinical Science, and Ward 4-East | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.7 | PROFESSIONAL: 0.8 | OTHER: 0.9 |
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| SUMMARY OF WORK (200 words or less - underline keywords) <p>The general purpose of this project is to investigate the roles of <u>autonomic nervous system activity</u>, <u>attention</u>, and <u>information processing</u> and their inter-relationships in the pathology, etiology, and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity as assessed by peripheral measures, such as <u>skin conductance</u>, <u>heart rate</u>, and <u>skin temperature</u>. Subjects with diagnoses of <u>schizophrenia</u>, <u>depression</u>, and <u>neurosis</u> are tested under conditions of rest, presentation of tones, and performance on <u>reaction time</u>, <u>mental arithmetic</u>, <u>two-flash discrimination</u>, or <u>tachistoscopic recognition</u> tasks. Biological mechanisms influencing ANS activity and attention are investigated by testing the effects of drugs and other treatments and by correlating these variables with enzyme activity and levels of biogenic amines and their metabolites. Psychological determinants are investigated by correlating the results with personality, mood, and personal history questionnaires by information from interviews, and by the effects of procedural variations.</p> | | |

Project Description

A. Objectives

The major objective of this project is the further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention, and their interrelationships in psychiatric disorders, primarily schizophrenia. The overall strategy involves studies of ANS and attentional relationships to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, "high risk" and personality studies in normal volunteers, and studies of the measurement of ANS activity.

B. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) usually measured bilaterally, heart rate (HR), vascular activity (skin temperature and finger pulse volume), and respiration while subjects are resting, exposed to a series of nonsignal tones of constant or of variable intensity and performing tasks. Tasks include tests of attention using reaction time techniques, tests of perceptual speed using two-flash discrimination and tachistoscopic recognition, and tasks designed to be moderately stressful. A mini-computer system is used to run the experiments and to collect and analyze the data. Studies in various stages of completion are listed below.

1. Schizophrenia Studies

a. A study with BPB (see Z01 MH 00132 BPB) of newly admitted, drug-free patients used a "balloon stress" (blowing up a balloon until it pops) and two tests of perceptual speed given on different days. The tachistoscope task allows separate evaluation of ANS responses to stimuli differing in significance and to positive and negative reinforcements. This should allow testing of the hypothesis, developed in previous studies, that schizophrenics' ANS does not respond appropriately to variations in stimulus significance. This study also includes several rest periods and a series of nonsignal tones for comparative purposes.

b. In current studies, ANS recording is being carried out in two sessions of rest, tone series, and reaction time using the first method described below. In addition, several methods of assessing attention deficits using reaction time (RT) techniques are being compared: (1) the classical "set" procedure of Shakow which involves variations in the foreperiod in a simple auditory RT paradigm, (2) RT to visual and auditory stimuli, measured when the stimuli are predictable, unpredictable, or simultaneous (but unpredictably so). We have confirmed previous findings in normals that although RT to tones is faster than RT to lights when the stimuli are predictable, RT to light is faster under unpredictable simultaneous presentation. This is taken to indicate an attentional bias toward visual stimuli or visual dominance, (3) comparison of ipsimodal vs. crossmodal sequences of tones and lights in a simple RT paradigm, plus occasional simultaneous presentation to assess "intersensory facilitation." Simple RT is faster under simultaneous presentation of a tone and light in the context of an unpredictable series presumably because the subject's response is triggered by whichever of the two stimuli he is attending to.

c. Patients are being tested during their hospitalization using a protocol of rest periods, a series of variable intensity tones (60-100 dB), and a two-flash discrimination procedure. Patients in this study are on an active treatment or placebo. Drugs, such as pimozide, lithium, naltrexone, GHB, and propranolol, and prazosine and other treatments, such as hemodialysis and plasmapheresis are evaluated.

2. Studies on nonschizophrenic psychopathology

a. Patients with depressive and obsessive compulsive disorders are being tested shortly after hospital admission or as outpatients (on a protocol identical to the first part of the schizophrenics' protocol described in l.b. above) in collaboration with the CNB. Patients are tested while being treated with the tricyclic antidepressant clomipramine and the Type A monoamine oxidase inhibitor clorgyline as in l.c. above. Adolescent obsessive-compulsive patients and aged-matched controls are also being studied in collaboration with BPB.

b. In collaboration with BPB, ten cases of multiple personality have been tested in 4-5 sessions each on short versions of the rest, tones, and RT time procedure. The method is to test the same three different personalities in a different order in each session to control for adaptation and compare the between-personality variance to the within-personality variance.

c. A study of women in different phases of their menstrual cycle has been initiated in collaboration with CNB and BPB. Women who report varying degrees of premenstrual discomfort, ranging from none to clinically diagnosable affective disorders are being studied. In addition to providing information on ANS involvement in premenstrual tension, we hope this study may help elucidate state and trait issues in ANS functioning in psychopathological conditions.

d. Men who had a diagnosis of early infantile autism are being tested with part of our standard protocol in collaboration with BPB.

3. Studies on normals

a. Two "high-risk" studies in collaboration with BPB (Project #Z01 MH 0035 BPB) in which subjects were selected on the basis of performance on attention tasks, have been carried out. In one, subjects were selected for very good or very poor performance on the Continuous Performance Task, and in the other, pendulum eye-tracking was used. The procedures we have used were similar to those used in the current studies on schizophrenia.

b. Two studies of reactions to physical and psychological stress have been done in collaboration with LCS and BPB. In addition to ANS measures, measurement of changes in norepinephrine, B-endorphin, and cortisol from plasma have been made.

c. In collaboration with LSES (Project #Z01 MH 00674 LSES), a method of confirmatory factor analysis is being used on ANS and personality data from 95 normal subjects. This has the objective of developing error-free measurement models of the structures of these systems, in order to reduce the large number of

variables generated by the ANS and personality assessment procedures to many fewer and more "pure" concepts. This method may lead to causal models of the interrelationships between systems.

C. Major Findings

1. Schizophrenic Studies

a. In a completed study, we showed that a pattern of ANS and attentional functioning - high ANS "arousal," small ANS responses, particularly to meaningful or demanding stimuli and situations, slow adaptation and habituation, and poor attention - characterized unmedicated acute schizophrenics compared to normal controls and within the schizophrenic group, predicted a poor clinical outcome of a 4-month hospitalization. Results from the newer study generally confirm those from the previous study for the schizophrenics as a whole compared to controls. An anomalous finding in the previous study - that skin conductance level, a commonly used index of ANS activation, differed from the other indices in being slightly lower in the schizophrenics - was also partially confirmed in that it was quite similar in the two groups. We are starting to analyze the relationships between the ANS data and symptoms, clinical course, and biologic markers. The presence or absence of Schneiderian "first rank" symptoms did not affect the ANS results in a major way, but we found that 8 patients with large sulci as measured from CT scans had significantly smaller ANS reactions to stimuli and tasks than 20 with normal CT scans. Patients without first rank symptoms who also had normal CT scans seem minimally deviant from controls on ANS measures.

b. Data are still being collected, but it is apparent that the phenomena of visual sensory dominance and intersensory facilitation found in normal subjects also occurs in schizophrenics.

2. Studies on nonschizophrenic psychopathology

a. Clinically, clomipramine was quite effective in reducing obsessional symptoms while clorgyline had minimal effects in most patients (see Project Z01 MH 00336-03 CN). Psychophysiologically, the two drugs had rather similar effects in reducing electrodermal base levels and responsivity, increasing HR and decreasing HR variability. However, clomipramine had generally somewhat more robust effects, especially on the cardiovascular variables. Clomipramine did seem to have unique effects in reducing skin conductance response amplitudes and there was some evidence of faster habituation of the orienting response only on this drug. If, under more detailed evaluation, this finding stands up, it may suggest a mechanism for the clinical effectiveness of clomipramine since slow habituation has been implicated in the etiology of the disorder. When drug-free, neither the obsessive adults nor children have shown, as a group, the labile and "aroused" ANS recordings that one would expect from a disorder that has a high anxiety component. Preliminary comparison of the adolescent patients with their controls has shown a reduced ANS response to task performance. Individual differences in ANS activity will be correlated with other biologic variables and with treatment response in both the adult obsessive and depressed patients.

b. Multiple personality subjects are being evaluated on a case-by-case basis. Preliminary analyses of the data suggest consistent differences in RT among personalities in a majority of the subjects and that habituation of the skin conductance orienting response in one personality may be unaffected by the prior experience of another personality.

c. & d. No reportable findings as yet.

3. Studies on normals

a. Poor attenders, as defined by the CPT, were found to have increasingly impaired reaction time as the amount of processing required increased from simple (20-25 msec) to choice reaction time (100 msec). This deficit seems due to a problem of shifting attention - from longer to shorter foreperiods in the Shallow procedure or between stimulus modalities in the other procedures. Thus, we have found a rather specific attention deficit in normal subjects that appears on several tests and is similar in kind to what has been found in psychopathology. Subjects with poor eye tracking were not greatly impaired in choice RT but were especially slow in responding when the timing of stimulus onset was uncertain. Thus, the CPT and eye-tracking tasks seem to be related to distinct kinds of attention impairments.

b. In both stress studies, it has been found that plasma norepinephrine increases markedly to physical stress, but minimally to psychological stress. Other purported indices of ANS activity, notably skin conductance and heart rate, change markedly to both types of stress. This suggests relatively greater peripheral control of norepinephrine and relatively greater central control over the other measures. Coronary prone (or "Type A") middle-aged men surprisingly evidenced higher base levels and smaller increases in norepinephrine and heart rate to physical (postural change from supine to standing) than Type B men.

c. Measurement models have been obtained successfully for concepts of skin conductance tonic arousal, skin conductance lability, skin conductance response speed and heart rate arousal for males and females in two different testing sessions, and for concepts of activity and mental health derived from a large number of personal history and personality variables. The strongest relationships found indicate that for women activity is negatively correlated with cardiovascular activity and for both genders perceptual speed is positively correlated with skin conductance indices of ANS arousal. Platelet monoamine oxidase activity correlates negatively with active behavior and positively with skin conductance activity.

Proposed course:

Analysis will continue of data for the completed project on schizophrenia with the goals of determining the relationship of ANS variables to diagnosis, diagnostic subtype symptomatology, severity of psychosis, performance on tests of attention and perceptual speed, degree of improvement during hospitalization and improvement on specific treatments. ANS activity in patient groups will be studied in relation to data obtained from biochemical assays of body fluids such as monoamines and their metabolites in CSF and monoamine oxidase activity. Predictors of clinical change after amphetamine infusion will be sought.

Collection of data will continue for current projects on schizophrenic and nonschizophrenic psychopathology. It is hoped to broaden the diagnostic comparisons by testing patients with severe anxiety neuroses. Normal controls will be tested on the same protocol. This protocol will be used in the collaborative LPP project on attention disorders.

Investigation of ANS and behavioral effects of various pharmacological therapeutic agents will continue for all these groups with the purposes of determining the comparative effects of the drugs and correlates with clinical response.

Data analysis will be completed on the high risk projects for group comparisons and correlation with other data on the same subjects. We will complete the general model of ANS task performance, personality, and mood variables to attempt to model other features of the data such as adaptation and habituation. If these methods prove useful, we will apply them to other data bases for both patients and normal subjects.

Significance to biomedical research and the program of the Institute:

Investigations of ANS activity and attention in psychiatric disorders, especially schizophrenia, have produced promising results which suggest that these processes may play fundamental roles in the etiology and expression of the disorders. Limitations on inferences to be drawn from measures of ANS activity come from incomplete understanding of their biological and psychological determinants. One of the main goals of this research is to increase this understanding by investigations of biological and psychological correlates and improving measurement techniques. Evidence of ANS effects of amphetamine in normal men, which are similar to the ANS activity seen in unmedicated schizophrenics (see Project #Z01 MH 00486-09), and similar effects of L-dopa reported in the literature permit the hypothesis that at least some aspects of ANS functioning may reflect the activity of dopaminergic systems. The dynamic nature of these measures permits the study of processes, such as adaptation, habituation, response to and recovery from stress, and effects of single stimuli through noninvasive techniques. Thus, further understanding of their mechanisms could greatly increase their utility in investigations of psychopathology. Continued investigations of the diagnostic specificity of these processes and of their relationships to other clinical features and to prognosis are necessary to confirm and extend our previous results and to test the limits of their generality.

Publications:

Rapoport, J.L., Elkins, R., Langer, D.H., Sceery, W., Buchsbaum, M.S., Gillin, J.C., Murphy, D.L., Zahn, T.P., Lake, C.R., Ludlow, C., & Mendelson, W. Childhood obsessive-compulsive disorder. Am. J. Psychiatry 138:1545-1554, 1981.

Zahn, T.P. Autonomic nervous system markers of diagnosis and prognosis in schizophrenia. In Hanin, I. and Usdin, E. (Eds.): Biological Markers in Psychiatry and Neurology. London, Pergamon Press, in press.

Bernstein, A.S., Zahn, T.P., Gruzelier, J.H., Patterson, T., Straube, E., Frith, D., & Venables, P.H. An analysis of skin conductance orienting response in samples of British, American, and German schizophrenics. Biol. Psychol., in press.

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|--|---|---------------------------------------|----------------------|-----------------------|-----|------|---------------------------|----------------|-----|------|--|--------------------------|--|--|---------------|--------------------|-----|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 00486-10 LPP | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Psychophysiological Concomitants of Minimal Brain Dysfunction in Children | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Theodore P. Zahn</td> <td style="width: 33%;">Research Psychologist</td> <td style="width: 15%;">LPP</td> <td style="width: 19%;">NIMH</td> </tr> <tr> <td>OTHER: Judith L. Rapoport</td> <td>Chief, Unit on</td> <td>BPB</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Childhood Mental Illness</td> <td></td> <td></td> </tr> <tr> <td>Robert Elkins</td> <td>Clinical Associate</td> <td>BPB</td> <td>NIMH</td> </tr> </table> | | | PI: Theodore P. Zahn | Research Psychologist | LPP | NIMH | OTHER: Judith L. Rapoport | Chief, Unit on | BPB | NIMH | | Childhood Mental Illness | | | Robert Elkins | Clinical Associate | BPB | NIMH |
| PI: Theodore P. Zahn | Research Psychologist | LPP | NIMH | | | | | | | | | | | | | | | |
| OTHER: Judith L. Rapoport | Chief, Unit on | BPB | NIMH | | | | | | | | | | | | | | | |
| | Childhood Mental Illness | | | | | | | | | | | | | | | | | |
| Robert Elkins | Clinical Associate | BPB | NIMH | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | |
| Biological Psychiatry Branch LAB/BRANCH | | | | | | | | | | | | | | | | | | |
| Laboratory of Psychology and Psychopathology SECTION | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.3 | PROFESSIONAL: 0.2 | OTHER: 0.1 | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | |
| <p>Many investigators believe that the <u>autonomic nervous system</u> (ANS) may be involved in <u>hyperactivity</u> (HA) in <u>children</u>. The objectives of our studies are to investigate differences in autonomic functioning between HA and normal children by means of peripheral indicators, such as <u>skin temperature</u>, and to assess the effects of the drugs on both autonomic activity and on task performance which depends on <u>attention</u> such as <u>reaction time</u>. The hypothesis that HA and normal children respond differently to stimulant drugs has been tested in a study of the effects of <u>d-amphetamine</u> on autonomic activity and attention in 6 to 13-year-old normal and HA boys. On the same protocol, male adults have been tested for age differences in drug effects. Studies have been done on the acute and chronic effects of <u>caffeine</u> on these measures in boys and in normal men.</p> | | | | | | | | | | | | | | | | | | |

Project Description

The general purpose of this project is to investigate the role of autonomic nervous system (ANS) activity and attention in hyperactivity in children and to study the effects of stimulant drugs on these processes in hyperactive and normal children and in normal adults.

Studies have been finished testing the effects of caffeine in boys and men using the same experimental procedures as were used in the amphetamine studies. The objectives of these studies are to compare the effects of these two "stimulant" drugs and, since caffeine has been reported to be a competitive inhibitor of the benzodiazepine receptor, to evaluate it as a possible pharmacological model of anxiety.

Results of these studies have been detailed in previous annual reports and the amphetamine studies have been published. Papers focusing on the attentional and ANS effects of caffeine in children and adults are in the planning stage. A new study on chronic caffeine use in children is being planned with Dr. Rapoport (See Project #Z01 MH 00153-05 BP) to correct some of the methodological difficulties in the previous one in which the effects of chronic caffeine consumption, acute withdrawal from caffeine in habitual users and personality traits leading to caffeine consumption were confounded. In the new study, subjects will be "on" or "off" caffeine for long enough periods of time to separate these factors.

Significance for biomedical research and the program of the Institute:

These studies are significant for biomedical research and the program of the Institute in several ways. First, the study of ANS changes after drug administration may help elucidate the mode of action of the clinical effects. Second, since the pharmacological effects of these drugs are partially understood, these studies can elucidate the mechanisms of ANS activity and help to interpret the ANS findings on clinical populations. Third, amphetamine abuse is a public health problem and caffeine abuse may be one, especially in children. Further understanding of the biological and psychological effects of these drugs may help in dealing with these problems.

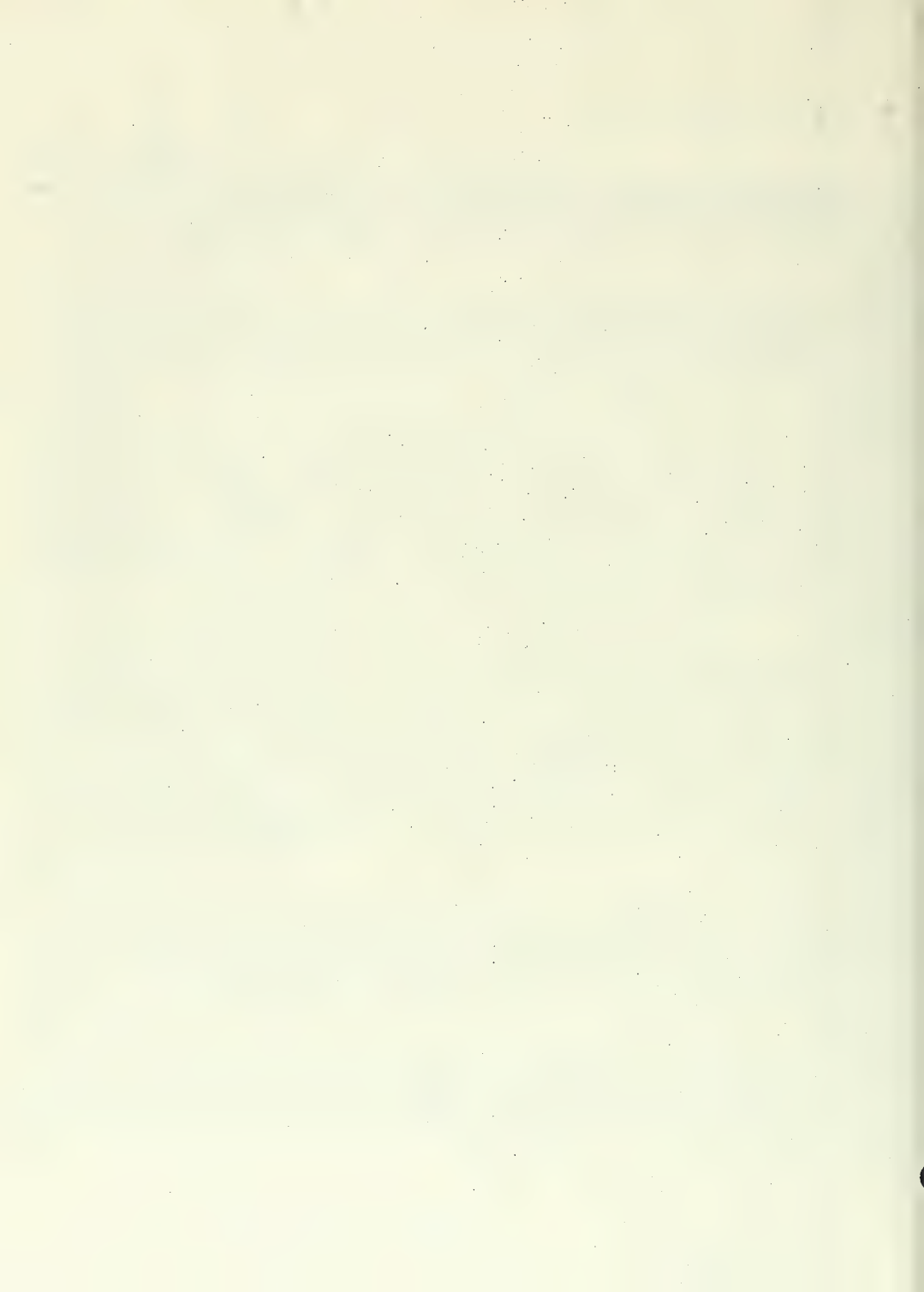
Proposed course:

In addition to the new study on chronic caffeine use in children described above, the future course of the project will include a detailed examination of the nature of the attention deficit in hyperactivity (now called "Attention Deficit Disorder" in DSM III) in line with the general program of this laboratory to develop a taxonomy of attention disorders.

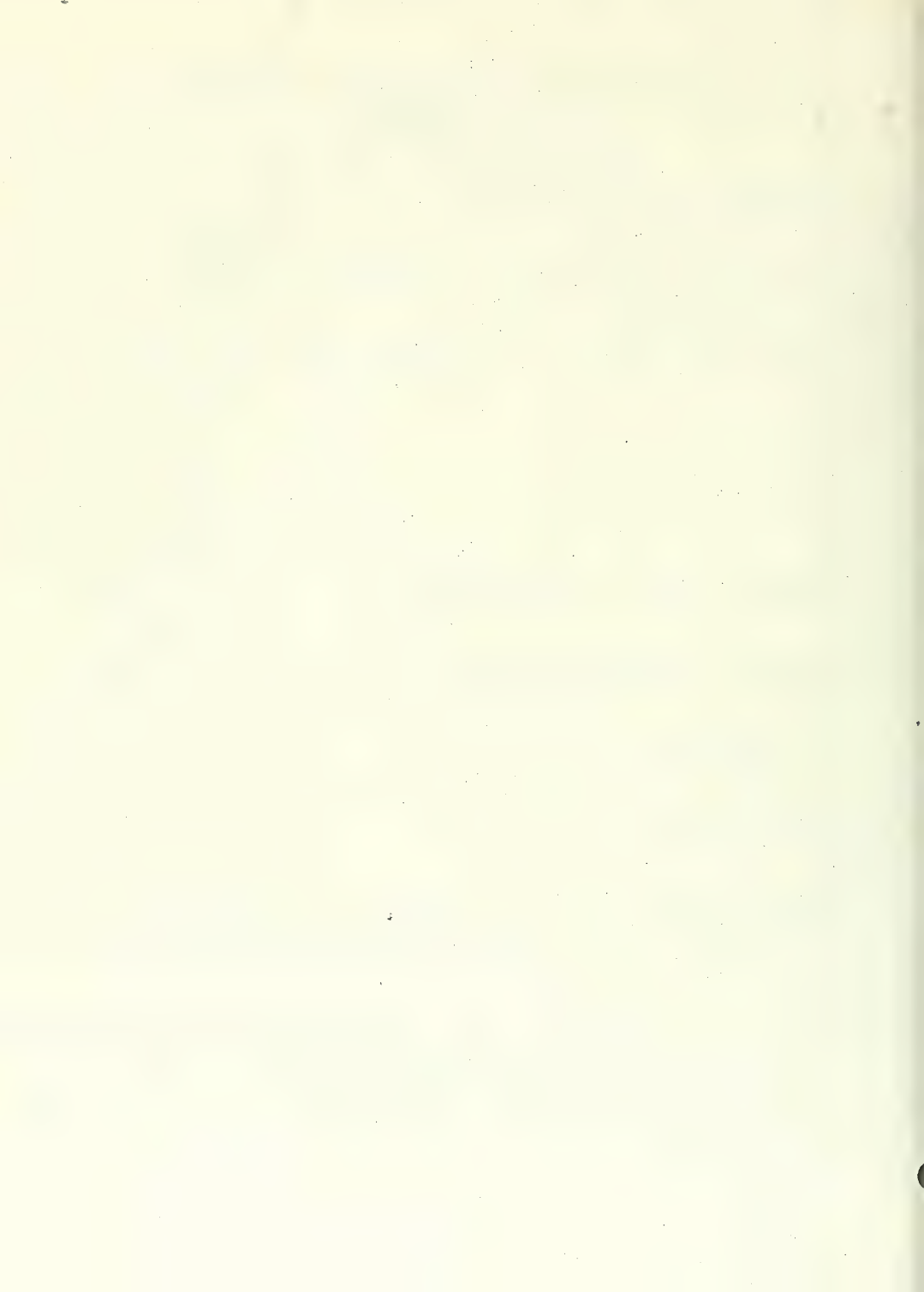
Publications:

Rapoport, J.L., Elkins, R., Neims, A., Zahn, T.P., and Berg, C.J.: Behavioral and autonomic effects of caffeine in normal boys. Developmental Pharmacology and Therapeutics. Basel: S. Karger, 1981, pp. 74-82.

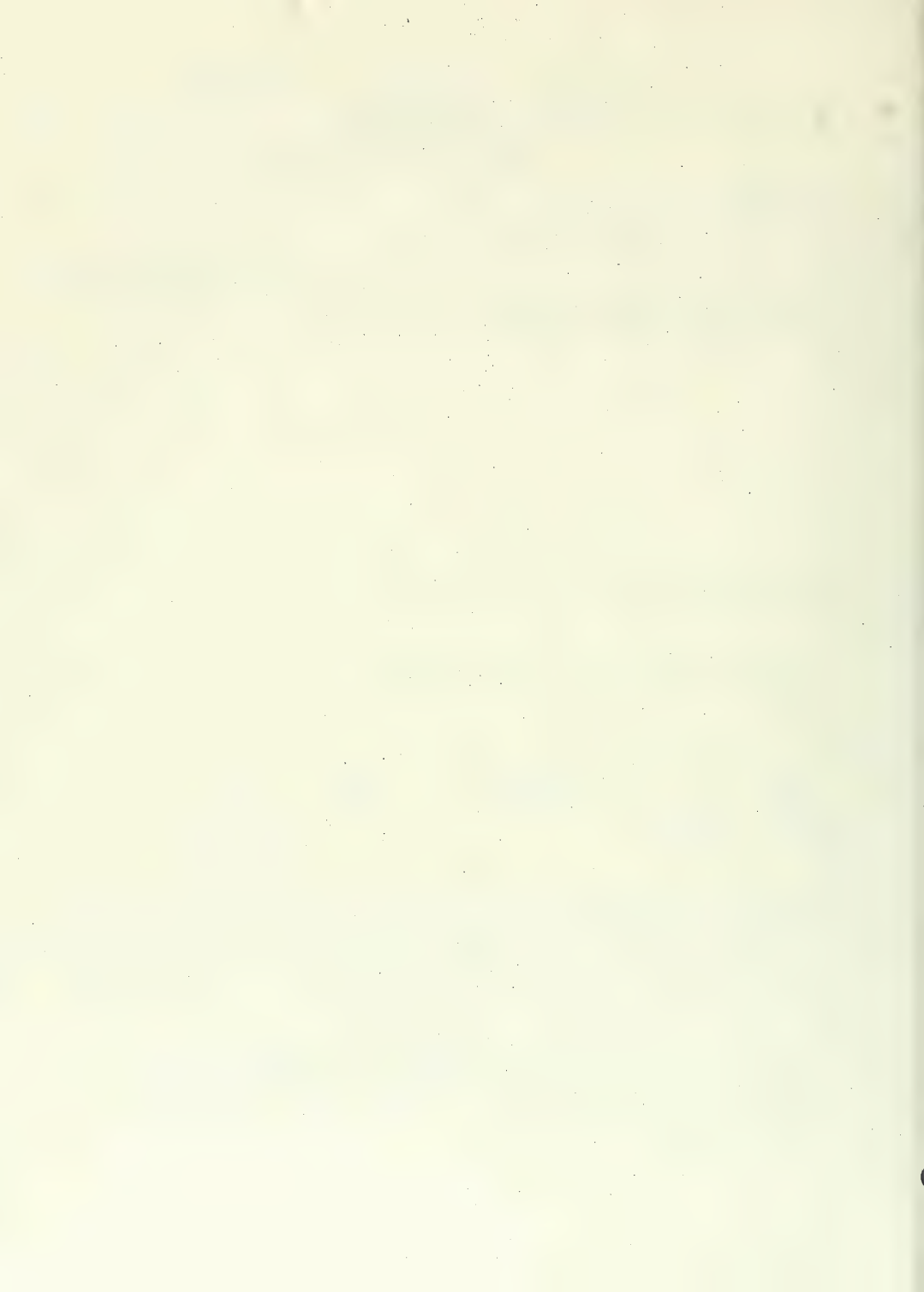
Elkins, R., Rapoport, J., Neims, A., and Zahn, T.P.: Behavioral Effects of Caffeine in Normal Boys. In Miller, S.A. (Ed.): Nutrition and Behavior. Philadelphia, Franklin Institute Press, 1981, pp. 167-176.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00488-17 LPP | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Individual Differences in Survival and Reproduction Among Old Colony Mennonites | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Gordon Allen</td> <td style="width: 33%;">Guest Worker</td> <td style="width: 33%;">LPP NIMH</td> </tr> <tr> <td>OTHER Calvin W. Redekop</td> <td>Professor of Sociology and Anthropology</td> <td>University of Waterloo and Conrad Grebel College</td> </tr> </table> | | | PI: Gordon Allen | Guest Worker | LPP NIMH | OTHER Calvin W. Redekop | Professor of Sociology and Anthropology | University of Waterloo and Conrad Grebel College |
| PI: Gordon Allen | Guest Worker | LPP NIMH | | | | | | |
| OTHER Calvin W. Redekop | Professor of Sociology and Anthropology | University of Waterloo and Conrad Grebel College | | | | | | |
| COOPERATING UNITS (if any) University of Waterloo and Conrad Grebel College | | | | | | | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, MD 20205 | | | | | | | | |
| TOTAL MANYEARS: 0.1 | PROFESSIONAL: 0.1 | OTHER: 0.0 | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This study was undertaken to seek evidence of <u>natural selection</u> with respect to human behavioral characteristics. The population chosen consisted of about 13,000 German-speaking <u>Mennonites</u> whose culture resembles in many respects that of pre-industrial European peasants. A census-survey was made in 1967 and vital records of the church were copied. For 569 families in the file, data include complete vital records and factor scores on several dimensions of <u>social and economic behavior</u> . Lengthy editing processes have now been completed and final analysis is in progress. This will first, specify parameters of <u>survival and reproduction</u> ; second, explore interrelations among vital events such as effect of infant mortality on birth intervals; third, examine the effects on fertility of such variables as wealth, medical care, and and social status. This project was terminated 10/18/81. | | | | | | | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00489-25 LPP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Variables Affecting Twin Birth Frequencies | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Gordon Allen Guest Worker LPP NIMH | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, MD 20205 | | |
| TOTAL MANYEARS: 0.1 | PROFESSIONAL: 0.0 | OTHER: 0.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Significant variations in the <u>twinning rate</u> can be observed both in the long term and in the short term. Attempts to explain such variation have been based variously on coital frequency, declining sperm counts, a fertility advantage in twin-prone women, variation in early loss of embryos, and other phenomena.</p> <p>Statistical findings in this project complement endocrinological research and implicate social and psychological influences on gonadotrophic hormone levels. Since the latter control estrogen and progesterone secretion, this phenomenon links <u>social events</u> to health and fertility in ways that will be explored further.</p> <p>This project was terminated 10/1/81.</p> | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00491-06 LPP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Personality factors and psychophysiological responses to changing stimulus input. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Theodore P. Zahn Research Psychologist LPP NIMH OTHER: Thomas N. Robinson, Jr. Guest Worker LPP NIMH | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 0.5 | PROFESSIONAL: 0.5 | OTHER: 0.0 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this project are to investigate relationships between differences in <u>personality</u> , <u>sensory thresholds</u> , and <u>autonomic nervous system</u> (ANS) activity in normal humans and to study <u>racial differences</u> in ANS activity. <u>Bilateral skin conductance</u> , <u>heart rate</u> , <u>vasomotor activity</u> , and <u>respiration</u> have been recorded in two sessions in which constant and variable intensity tones and lights are presented, and auditory and two flash thresholds determined by methods which permit signal detection analyses. Several standardized personality tests were also given. The procedures allow determination of the effects of <u>stimulus intensity</u> and <u>heteromodal stimulation</u> on ANS activity. The protocol allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity, and personality, some of which have implications for the etiology of psychopathology. | | |

Project Description

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion," "sensation seeking," and "field dependence," each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships between personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., over-reactive to weak stimulation and under-reactive to strong stimulation - "transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, and ANS activity in normal humans. Other objectives are to assess racial differences in ANS activity and in its relationships to the other variables in the study and to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality.

Over 180 normal volunteers have been assessed on several personality dimensions, including the Eysenck scale of extraversion, neuroticism, and psychoticism, field dependence, sensation seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance and given tests of ANS and sensory functioning in two separate sessions as described earlier.

Results reported last year showed that contrary to the prediction that introverts should show a transmarginal inhibition, introverts compared to extraverts had large ANS reactions to high intensity auditory stimulation. This finding may have some significance for psychopathology since a review of the literature on the psychophysiology of schizophrenia in preparation has revealed that the apparently incongruous results of studies on the electrodermal orienting response are consistent with the generalization that unmedicated schizophrenics overreact to intense stimulation and underreact to weak stimulation compared to normal controls.

Subjects scoring high and low on a scale of "psychoticism" differed in the direction of the relationships between ANS arousal and two-flash threshold (TFT) and sensitivity. These are similar to results found in comparing schizophrenic and normal subjects.

In a current study, we are exploring the effects on TFT of manipulating arousal using a postural change which we have found to double plasma norepinephrine and to increase cardiovascular and electrodermal base levels. Subjects are tested in a reclining position in one session and standing in another. This simple method

should have minimal direct effects on attention or distraction unlike many other methods which have been used. The prediction is that subjects differing in psychotocism should differ in the effects of the postural change on TFT measures. Several other questions can be investigated such as differential effects on bilateral electrodermal measurements and effects on the orienting response.

Analysis of this large data base is continuing. In addition to further tests of personality theories and laterality differences, racial differences are being studied. Much previous research has found black subjects to be less reactive than whites on electrodermal measures. This has usually been attributed to peripheral (skin) factors. Our data has confirmed these findings, but we showed lower cardiovascular reactivity in black subjects, as well suggesting a more central locus for the difference or perhaps a difference in the psychological approach to the experiment. These findings were reported at a scientific meeting last year.

Significance for biomedical research and the program of the Institute:

Further understanding of how autonomic, perceptual, and personality variables interact in normal subjects should be of great assistance in interpreting the autonomic and perceptual results from studies on psychopathology in which similar methods are used in our other studies. Similarly, the study of racial differences in normals will help us evaluate the results of racially mixed samples of patients. This project has been very useful in the development of protocols for studies of psychopathology.

Proposed course:

The sample size of the postural change study will be increased to permit testing of the effects of individual differences in personality. Analysis of the older data will continue with special emphasis on lateral differences in electrodermal activity. There is much confusion in the literature about the interpretation of such differences, but there are some interesting findings in psychiatric patients. Since this is one of the few studies in the literature to test a large sample of left-handed subjects, the data should be of value in such interpretation.

Publications:

None.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00495-06 LPP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Psychobiology of Cognitive Processes | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Herbert Weingartner OTHERS: Michael H. Ebert J. Christian Gillin Philip Gold Dennis Murphy Elizabeth Parker Robert Post Judith Rapoport Stanley I. Rapoport Edward Silberman Richard Stillman Allan F. Mirsky | Chief, Unit on Cognitive Studies Chief, Sec. Exp. Therapeutics Research Psychiatrist Chief, Unit on Neuroendocrinology Chief, Neuropharmacology Branch Senior Staff Fellow Chief, Section on Psychobiology Chief, Unit on Childhood Mental Illness Chief Staff Psychiatrist Research Psychiatrist Chief | LPP NIMH LCS NIMH BPB NIMH CPB NIMH CNB NIMH LCS NIAAA BPB NIMH BPB NIMH LN NIA LPP NIMH SMRC NIDA LPP NIMH |
| COOPERATING UNITS (if any) | | |
| Biological Psychiatry Branch, DCBR, NIMH Laboratory of Clinical Science, DCBR, NIMH Clinical Neuropharmacology Branch, DCBR, NIMH | NI Alcohol and Alcohol Abuse National Institute on Aging National Institute on Drug Abuse Clinical Psychobiology Branch | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.0 | PROFESSIONAL: 1.8 | OTHER: 0.2 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less. - underline keywords) We are investigating the <u>psychobiology</u> of cognition in man. We attempt to inter- relate psychological and biological determinants of various components of <u>cognition</u> , such as the specific and discrete psychobiological mechanisms that define the <u>acquisition, processing, encoding, consolidation, and retrieval</u> of experience. Experiments are designed to examine the biological and psychological determinants of psychiatric and neuropsychiatric alterations in cognitive processes in adults and children. Specific forms of central nervous system dysfunctions (e.g., as defined by type of lesion in neuropsychiatric disorders) may affect specific and distinct components of cognitive processing. Similarly, psychoactive drugs that affect discrete aggregates of neurons may affect discrete aspects of cognition and information processing. Based on empirical studies of clinical populations (e.g., depression, Alzheimer's disease, Korsakoff's disease, forms of learning impairments in children) and on several types of psychoactive agents (cholinergic drugs, noradrenergic drugs, neuropeptides), it has been possible to begin to describe the <u>psychobiological</u> relationships between <u>semantic</u> and <u>episodic</u> memory, encoding processes, and effortful (active) cognitive operations as opposed to automatic cognitive processes. | | |

Project Description:

The research projects reviewed here are all concerned with the psychobiology of cognitive processes in man. They have been designed to explore the psychological and biological determinants of various aspects of cognitive processes and their interrelationships. Studies have examined the psychobiological processes that define the encoding, processing, learning, and storage of information, how processed events are altered, or elaborated in memory, the consolidation and retention of information, and the mechanisms that are involved in retrieval of stored information. Some of the components of cognitive processes that have been examined include attentional determinants, aspects of short-term memory, the consolidation of information, in long-term memory, the kinds of state or trait specific cognitive strategies, "effortful" vs. automatic cognitive processes, and the kinds of strategies that subjects use to retrieve experience that they have stored in memory. Recent studies have also focused on the distinction between the psychological and biological determinants of episodic and semantic (knowledge) memory.

Two broadly defined types of strategies are used to define the psychobiology of cognitive processes. One is to contrast the effects of different treatments on different components of cognition, such as on attention, information storage, consolidation, encoding (automatic and effortful), and retrieval processes. These studies include: (1) pharmacological manipulations, such as cholinergic drugs, noradrenergic drugs, abused drugs (alcohol, marijuana), central nervous system depressants, neuropeptides, and (2) behavioral manipulations that alter reinforcing properties of stimulus arousal/activation, stimulus attributes (altering encodability), and types of stimulus processing strategies subjects use to process information. These studies are carried out in unimpaired subjects, as well as in patient groups with different forms of psychology. Would different kinds of pharmacological or behavioral pathology manipulations of cognition lead to different forms of enhanced or disrupted cognition? Contrasting these different treatment effects on different CNS systems and relating these changes to cognitive responses should be particularly useful in providing us with a picture of the structure of the psychobiology of cognition. A second type of broadly defined alternative strategy for researching the psychobiology of cognition is to contrast systematically different forms of cognitive failures as seen in different psychiatric and neurological syndromes. Would the disrupted cognition seen in some psychopathological states be qualitatively and quantitatively different and related to specific changes in central nervous system activity? For example, how are the amnesic/cognitive impairments seen in Huntington's disease, Korsakoff's syndrome, and Alzheimer's disorder different? How might the differences be an expression of the specificity of central nervous system involvement in each of these disorders? In some instances, the possibility of discriminating between the form of the cognitive impairment is necessary for both adequate diagnosis and effective treatment, i.e., such as the cognitive disruptions that are part of depression as opposed to that produced by a progressive dementia. Frequently, depression is an integral part of a progressive dementia, and the cognitive impairment is a joint product of the two disorders. Some studies have also investigated the therapeutic potential of various psychoactive drugs and behavioral treatments. Do these attenuate or reverse the cognitive disruptions seen in various forms of

dementia, hyperactivity, and learning disability syndromes in children, depression, mania, and the schizophrenias? Each of the studies is clinically relevant as well as pertinent to understanding unimpaired cognitive processes.

In summary, all of the parts of this project are concerned with defining the discrete psychobiological components of cognitive processes that are involved in the appreciation, storage, retention, and retrieval of experience. The studies are concerned both with the bases of cognitive processes as well as clinical studies of disordered cognition.

1. Semantic (knowledge) memory and its relationship to other forms of learning and memory (episodic memory)

Studies have been designed that would begin to describe how knowledge is represented in memory (semantic memory) and how it would be accessed and used in order to encode or appreciate ongoing events. The relationship between semantic memory and episodic memory, how these are altered by various biological treatments and in neuropsychiatric disorders represents a very new and important area of investigation in exploring the psychobiology of cognition.

2. Pharmacological alterations (enhancement and disruption) of cognitive processes

These studies have contrasted the cognition enhancing effects of cholinergic agents such as arecoline, physostigmine, THA, and lecithin treatment with those of amphetamine and neuropeptides in both unimpaired subjects, as well as in patients (hyperactive children, Korsakoff's disease, Alzheimer's disorder, depressed patients). Do drug treatments that affect the central nervous system in different ways produce systematically different changes in cognitive processing? Are enhancements or disruptions of cognition determined through different psychobiological mechanisms, and might the cognitive response to different drugs make such a pattern discernible? Some neurotransmitter antagonists have also been used to model forms of impaired cognition in man.

In some instances, drugs that might disrupt aspects of the acquisition of information may enhance some other stage of cognitive processing (e.g., process-specific effects of drugs that alter cognition) can be used to better define a psychobiology of information processing in man. This research has also involved attempts to find clinically useful strategies for altering disrupted cognition in man.

3. State-dependent learning

This area of research provides a useful framework for exploring: (a) the qualitatively unique manner in which events are stored (encoded and retrieved from memory), (b) studies of disturbances in mood state and how these define mood-specific strategies for processing experience and remembering past events in memory, (c) qualitative changes in cognition in response to psychoactive drugs, (d) contextual factors as determinants for defining the nature of trace events in memory, (e) individual differences in susceptibility to state-dependent or dissociative mood/drug effects.

4. Memory consolidation

This research has focused on the psychobiological events that follow the acquisition (storage) of information and which occur well before processed information is to be retrieved from memory. Studies in both normal subjects and patients have examined the form and strength of stored information in memory and the processes that might further sustain, enhance, or disrupt stored trace events that are already part of memory. The rate of decay of information and the susceptibility of information to interference and to biological factors, may be important determinants in defining what is available and accessible in recall, once information has been stored in memory. Disruptions in consolidation may contribute to the cognitive failures in the dementias.

Drugs that disrupt memory and learning may do so by altering biological operations that succeed acquisition or learning. Likewise, drugs (e.g., neuro-peptides) may enhance aspects of learning and memory by facilitating the consolidation of learned information.

5. Behaviorally-defined mechanisms that alter components of cognition

Characteristics of stimuli such as: (a) organizational properties, (b) imagery, (c) emotional arousing attributes of stimuli, d) information presentation rate, (e) mode of processing, (f) language vs. pattern information, and (g) types of learning have been studied in relation to its effects on attention, acquisition (learning), strength of learning, retention, and components of information retrieval. The studies have examined these factors in normal controls, as well as in patient groups (depressed patients, hyperactive children, learning disabled, patients suffering from various forms of dementia). Some of the issues raised in these studies include the following: How might aspects of information processing alter the attentional, short-term memory, encodability, retention, and retrieval of information? Would different forms of psychological manipulations systematically alter different aspects or components of cognitive processes? Do patients who demonstrate failures to learn and remember do so because of disruptions in some, but not all of these component cognitive processes, and can manipulations of some characteristics of information processing change these disruptions in cognition? Recent studies have also begun to explore "effortful" vs. "automatic" information processing and how these are altered under different motivation/arousal conditions. This approach to cognition has been used to define the effects of depression on cognition, determinants of cognitive failures in the learning-disabled child, and drug-altered changes in cognition (see below).

6. Cognition and mood

Studies have included research of mood-related changes in: (a) the brain lateralization of cognitive functions; (b) arousal and activation and its role in information processing; and (c) the encoding and retrieval of events in normal mood and in depression or mania. This research has examined how patients with disturbances in mood process information in a mood-state specific manner. In addition, this research has begun to examine mood-related changes following psychoactive drug treatment and its interactive role in altering cognitive

processes. Other research has explored the degree to which effortful processing of information is compromised as a motivation-related determinant of thinking in depressed patients.

7. Mechanisms of cognitive impairments that determine forms of learning disabilities

Studies have been designed which investigate: (a) forms and incidence of various kinds of learning disabilities in children; (b) the descriptive nature of the learning disabilities in these children; (c) potential strategies for their remediation.

Methods

Three strategies have been used in these studies. One involves manipulation of different biological systems that may play a role in different aspects of cognition in man. Various neurotransmitter agonists and antagonists, as well as agents that affect neuroendocrine functioning are contrasted to one another in both impaired and unimpaired subjects. A second strategy involves systematic comparison of various forms of cognitive failures apparent in different clinical groups. These methods use neuropathological, neurochemical, and neuroanatomical changes that are apparent in different forms of chemical syndromes to provide a matrix of the biological determinants of these different forms of impaired cognition. The third set of methods involves systematic manipulation of stimulus, retention processing, and retrieval conditions.

A large variety of cognitive strategies has been designed to explore the psychological components of cognition in the studies that are part of this project. These strategies have included modified forms of current methods used in human information processing research, as well as newly-developed tools that might more adequately examine determinants of cognition in clinical studies and those assessing cognitive drug effects. These strategies have been developed in a number of studies and include measures and manipulations of: (a) organization of information; (b) informational context for processing information; (c) stimulus attributes such as imagery, emotional properties, frequency; (d) forms of learning and recall (free recall, prompted free recall, recognition memory, cued recall, serial learning, paired associates learning); (e) processing time and type of presentation of information; (f) type of processing strategy (processing on the basis of meaning or sound properties); (g) immediate vs. delayed recall of information with or without rehearsal of stored information; (h) presentation of language vs. pattern information to left vs. right hemisphere (methods used to investigate lateralization); (i) rapid (tachistoscopic) presentation of information; (j) measurement and manipulation of different forms of retrieval of information in memory, including forms of free recall, prompted or cued recall, recognition memory, method of "savings"; (k) very long-term memory retrieval; (l) assessment of effortful and automatic cognitive processes; (m) arousal and motivation in information processing.

Most recently new cognitive methods have been developed which also permit us to examine characteristics of semantic (knowledge) memory in contrast to the methods described above which are primarily useful for describing episodic

memory. These methods allow us to measure the structure of knowledge in memory and how readily it can be accessed and used in transforming events. This is being accomplished through the development of new behavioral techniques as well as psychophysiological and neurobiological methods (P300, positron emission methods, versions of average evoked response methods).

Findings: Psychobiology of Cognition

1. Cognitive changes in depression:

The pattern and determinants of cognitive changes in depression have been shown to be distinguishable from those expressed in other disorders (particularly in early stage progressive dementia). Depressed patients demonstrate a type of disordered thinking, one that is manifest in an inability to accomplish focused, detached analysis of information leading to impairments in concept learning, acquisition of information, and memory. This may be related to alterations in the cerebral lateralization that is involved in processing language in non-language information (a demonstrated right hemisphere advantage where normal is seen as left hemisphere advantage in processing information).

2. Semantic memory or episodic memory:

A series of studies has shown that semantic memory (knowledge memory) and episodic memory are psychobiologically distinct but interrelated types of information processing systems. We have shown that (a) semantic memory failures effectively characterize the cognitive defect in progressive dementia, (b) that episodic memory failures are determined by semantic memory impairments in progressive dementia patients, (c) other amnesic syndromes (such as in Korsakoff's disease) are due to different psychobiological determinants, other than those that affect access to semantic memory, and (d) neuropeptide treatments such as with arginine vasopressin may facilitate access to semantic memory. We have also shown that some aspects of information processing are particularly susceptible to disruption in depression. We have verified that depressed patients are impaired cognitively to the extent to which cognitive processes or operations require effortful rather than passive or automatic kinds of operations. The depressed patient, unlike progressive dementia patients, does not demonstrate impairments in learning and memory on automatic processing tasks. In addition, the extent to which these depressed patients are able to sustain effort is highly correlated with the intensity of their depression and likewise is highly correlated with the extent to which they can perform these non-automatic active cognitive operations.

It is also clear that the extent to which depressed patients are provided with organization or structure in an information processing task is the extent to which they are relatively indistinguishable from normal controls in terms of learning memory functions. When processing random information, depressed patients demonstrate profound cognitive deficits. On structured tasks, these deficits are attenuated. This finding has been particularly important in distinguishing between the cognitive dysfunction of depression and progressive dementia.

3. Cognitive impairment in progressive dementia and Korsakoff's disease and possible treatment strategies:

Recent findings from our laboratory have defined some of the characteristics and determinants of the cognitive dysfunction in progressive dementia patients. We know that information is relatively rapidly lost from memory, immediate memory is relatively unimpaired, and any type of learning-memory operation that requires the establishment of permanent trace events in memory is dramatically disrupted. Memory failures are, in large part, due to processing or acquisition deficits which then result in weak trace formation and therefore failures to retain information in memory. A considerable body of research has suggested a distinction between semantic memory and the repository of information of knowledge structures from episodic memory, i.e., memory for ongoing recent events. Although these two kinds of memories have been traditionally viewed as being separate and distinct, we have found an important link between the two. Based on recent findings relating these two systems, it has been possible to account for many aspects of the memory impairment in progressive dementia patients. In a series of studies, we have been able to demonstrate that the extent to which Alzheimer's patients have access to structures in semantic memory is the extent to which they are relatively unimpaired on many tasks of learning and memory. These results have important implications both diagnostically in distinguishing this group of cognitively impaired patients from other groups (e.g., cognitively impaired depressed patients) as well as for potential treatment strategies.

Most recently we have been able to show some facilitation of learning and memory in these patients, using two very different strategies. Cholinergic drugs seem to produce small improvements in learning and memory but only in those patients that are least cognitively impaired. In contrast, arginine vasopressin facilitates learning and memory by facilitating access to semantic memory (a mechanism of action that is consistent with the determinants of the memory failure in these patients).

Although Korsakoff patients are often as memory impaired as progressive dementia patients, the cognitive and biological determinants of their impairments are quite different. Unlike progressive dementia patients, the Korsakoff amnesia patient responds to attributes of stimuli that would ordinarily aid encoding such as (1) repeating information, (2) organizing information, (3) presenting pictures rather than words. Furthermore, the Korsakoff patient can learn procedures and remember them for very long periods of time. This is because unlike progressive dementia patients the Korsakoff patient is able to access semantic memory.

The findings have suggested that cognitive failures in progressive dementia are distinguishable from those evident in depression and other syndromes. This has prompted active study of drug and other treatment strategies for reversing such cognitive failures. By understanding both the mechanisms of cognitive impairments and the neurochemical response following various forms of drug treatment, it should be possible to design studies that would examine the therapeutic potential of various types of drug treatments. The mechanisms and determinants of the cognitive impairments in depression and dementia have

allowed us to devise strategies to distinguish clinically between these two groups. Characteristics of automatic versus effortful processing, the extent to which effort is extended in accomplishing tasks, and the processing of unrelated vs. related events allows us to distinguish clinically between the cognitive impairment in depression and that in the progressive idiopathic dementia patients.

4. Learning disabilities in children:

Drug treatments, such as stimulants, appear to facilitate learning and memory in some types of learning disabled children. These cognitive effects are seen primarily in those processes that require sustained effort. These effects are apparent and independent of other clinical changes in these amphetamine-treated children. In addition, learning that occurs in the amphetamine-treated state does not appear dissociated when remembering takes place in the untreated state. This is not like the kinds of dissociative, state-dependent, learning and memory effects that are seen in stimulant treated adults. These results are also important in considering the effects of stimulant treatment on the educational experience of learning disabled or hyperactive children. In a series of studies, we have attempted to describe the components of cognitive changes that are apparent in children with various forms of learning disability. We have examined two groups of these children, one where hyperactivity is part of the syndrome, and a second group where there is no evidence of hyperactivity or generalized retardation. Nevertheless, these children demonstrate dramatic impairments in learning and memory that resemble the kinds of disruptions in cognition that are evident in some groups of adults. The resemblance is closest to depressed patients; it also resembles the kinds of cognitive changes that are produced by drugs that disrupt cholinergic and noradrenergic activity. Both hyperactive and learning disabled children show impairments in effortful processing of information; automatic processing is left relatively intact. On incidental learning paradigms, these children are indistinguishable from normal controls. Both groups of children also demonstrate impairments in those characteristics of cognition that require the imposition of organization in memory. In many ways, the results we have obtained to date would suggest that the type of cognitive impairment seen in these children resembles that seen in depressed patients in contrast to the pattern of cognitive impairments evident in progressive dementia patients. The kinds of cognitive impairments are also like those that are apparent when unimpaired subjects are treated with drugs that disrupt or block catecholamine activity.

5. Neuropharmacological studies of cognition in man:

We have been able to demonstrate both in patient groups as well as in unimpaired subjects that the effects of cholinergic antagonists and agonists produce cognitive changes that are qualitatively different from those of drugs that have their major effect on catecholamine activity. There appears to be further specificity and distinctiveness in the role of neuropeptides such as synthetic vasopressin-like substances, and of naloxone, in determining aspects of learning and memory in both cognitively impaired patients (depressed patients and progressive dementia patients) as well as in unimpaired subjects. Different neurotransmitter systems and different kinds of neurochemical mediators are

involved in the regulation of various aspects of episodic memory (acquisition, retention, and retrieval of information) and other biological determinants which affect semantic memory. Furthermore, effortful cognitive operations appear to be determined by different biological mechanisms than those involved in automatic cognitive operations.

We have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and in the storage and retrieval of information. Scopolamine treatments which disrupt cholinergic activity produce an impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine-induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated progressive dementia patients.

In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in a drug free state, and are treated afterwards with arecoline, there is a comparable facilitation of information later recalled, when compared to learning and recall that occurs under arecoline treatment conditions.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input processing under drug state conditions. Unlike cholinergic manipulations, amphetamine appears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (size of the pool of trace events in memory). In a series of studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition in depressed patients, hyperactive children, normal children, and normal adults. Amphetamine also induces a change in state which serves as a state-specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state-dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine and the cognitive disrupting effects produced by scopolamine vs. lithium have served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

While alcohol has been viewed traditionally as one type of pharmacological manipulation that reliably produces learning and memory impairments in man, recent work from our laboratory in collaboration with NIAAA has demonstrated that post-processing manipulation including treatment with alcohol can in fact produce some enhancements in learning and memory. The focus on the biological and psychological events that follow the initial acquisition of information has generally been ignored in studies of cognitive processes. This consolidation phase of memory and the biological events that occur during this time may be important in establishing permanent records of experience. This is evident both in our studies using alcohol, with the paradoxical enhancement of what has been stored in memory and in the effects of vasopressin on reversing retrograde amnesia following ECT administration. Alcohol, when administered after the processing of information, produces an enhancement in recall when tested in the unintoxicated state. This effect has been seen as one that alters memory

consolidation. Most recent findings suggest that alcohol induces a brief excitatory phase (possibly mediated by changes in catecholamine activity) which affects memory consolidation. This excitatory phase may be important in defining some of the reinforcement properties of alcohol. This paradoxical cognitive facilitating effect of alcohol, administered during a consolidation phase of memory appears to highlight the differentiated mechanisms and components that make up information processing, memory, learning, and retrieval.

We have now completed a series of cholinergic trials in Alzheimer's patients and have demonstrated that cholinergic antagonists such as scopolamine mimic many of the characteristics that are evident in progressive idiopathic dementia, (Weingartner, et al., in preparation for Memory and Cognition). We have also noted that combinations of cholinergic agents do in fact produce small but reliable enhancements of some aspects of learning and memory in patients with Alzheimer's disease. The limiting factor here has been that the extent to which an enhancement in learning and memory is evident is largely a function of the degree of cognitive intactness of the patients.

Summary

The recently completed programmatic-research efforts of the Unit on Cognitive Studies has extended our knowledge of the psychobiological structure and determinants of cognition. Completed research has been valuable in better defining disordered mood, the nature of the information processing impairments in progressive dementia, Korsakoff's disease, and other alcohol-related disorders, and the nature of cognitive defects in learning disabled children. Neuropharmacological studies in both unimpaired subjects and patient groups has provided us with further information about the neurochemical events important for learning, memory, and cognition. These studies have also provided new approaches in the treatment of various forms of cognitive disturbances.

Specifically, we have begun to describe the major psychobiological differences and relationships between recent (episodic) memory and knowledge (semantic memory). This distinction between these two types of memory systems is the key to understanding forms of cognitive failure in man. We have developed a way of characterizing the nature of the cognitive changes in depression and how they determine learning and memory changes associated with disordered mood. It has been possible to model forms of cognitive impairments in man such as those that are seen in Alzheimer's disease, in drug studies of unimpaired subjects (cholinergic antagonists). This type of research has helped us in our efforts to facilitate aspects of cognition in these patients, using cholinergic agonists. Neuropeptides have also been used to treat some of these cognitive disorders (e.g., arginine vasopressin) and to model disorders of information processing in unimpaired subjects (with the use of naloxone).

Based on these research efforts, it seems important that we focus new efforts in exploring: (a) mediational processes that are involved in transforming and encoding information (using psychophysiological and neuropharmacological tools); (b) the relationship between the reward system and memory processes particularly as these would alter memory consolidation; and (c) new ways of facilitating impaired cognitive processes.

Significance to biomedical research and to the program of the Institute:

These research efforts have a direct bearing on how diagnoses of cognitive dysfunction are accomplished and the directions of future efforts for treating the cognitive impairment associated with a wide variety of psychiatric and neuropsychiatric disorders.

Proposed course:

We hope that current studies will lead to better diagnostic tools and effective therapies for cognitive dysfunction.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 00500-03 LPP | | | | | | | | | | | | | | | | | | | | | | | | |
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| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">Edward K. Silberman</td> <td style="width: 40%;">Clinical Associate</td> <td style="width: 15%;">LPP</td> <td style="width: 15%;">NIMH</td> </tr> <tr> <td>Herbert Weingartner</td> <td>Chief, Unit on Cognitive Studies</td> <td>LPP</td> <td>NIMH</td> </tr> <tr> <td>Robert M. Post</td> <td>Chief, Section on Psychobiology</td> <td>BPB</td> <td>NIMH</td> </tr> <tr> <td>John I. Nurnberger</td> <td>Senior Staff Fellow</td> <td>BPB</td> <td>NIMH</td> </tr> <tr> <td>Roger J. Porter</td> <td>Chief, Epilepsy Branch</td> <td>NDP</td> <td>NINCDS</td> </tr> <tr> <td>Steven D. Targum</td> <td>Chief, Evaluation Unit, The Psychiatric Institute, Washington, D.C.</td> <td></td> <td></td> </tr> </table> | | | Edward K. Silberman | Clinical Associate | LPP | NIMH | Herbert Weingartner | Chief, Unit on Cognitive Studies | LPP | NIMH | Robert M. Post | Chief, Section on Psychobiology | BPB | NIMH | John I. Nurnberger | Senior Staff Fellow | BPB | NIMH | Roger J. Porter | Chief, Epilepsy Branch | NDP | NINCDS | Steven D. Targum | Chief, Evaluation Unit, The Psychiatric Institute, Washington, D.C. | | |
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| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 2.0 | OTHER: 1.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <p> The purpose of this project is to investigate the <u>cognitive and perceptual changes</u> which are present in, and characteristic of, <u>major affective illness</u>. The present investigation comprises five separate ongoing studies: (1) <u>psychomotor</u> and <u>psychosensory symptoms</u> in affective illness, patients with complex partial seizures, and patient controls; (2) perception and recall of <u>emotional</u> and <u>neutral stimuli</u> in depression; (3) <u>hypothesis testing</u> in depression; (4) <u>lateralized hemispheric function</u> in depression; (5) relationship of <u>cognitive dysfunction</u> to <u>diagnostic subtype</u> and <u>neuroendocrine abnormalities</u> in depression. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description

The premise of this project is that there are, in addition to the well-known mood changes, important alterations in perceptual and cognitive processes in major affective illness. Such symptomatology may have important implications for the pathophysiology of affective disease and may provide significant contributions to delineating clinically and prognostically homogeneous subtypes of this condition. The present study is composed of eight investigations, looking at various aspects of the problem. They are aimed at documenting the nature and extent of cognitive and perceptual changes at the clinical level, and of investigating the degree and structure of such deficits in the laboratory. Below is a summary of the current studies within this project.

1. Psychomotor and psychosensory symptoms in affective illness.

A wide range of behavioral and perceptual manifestations, many with localizing significance in the brain, have been described as concomitants of complex partial seizures. The literature and clinical observation suggest that there may be areas of overlap between these phenomena and symptoms of major affective illness. The purpose of the present study is to investigate the nature and degree of such overlap. In collaboration with Dr. Robert Post, a structured interview has been devised to elicit presence of symptoms related to perception, thought processes, orientation, memory, and involuntary motor behavior. The interview is based on a survey of the literature describing such changes in epilepsy. Design of the project involves administering the interview to 120 patients; 40 are patients with a history of major affective illness currently being treated by units of the Biological Psychiatry Branch; 40 are patients with a firm diagnosis of complex partial seizures, under the care of the Epilepsy Branch, NINCDS (in collaboration with Dr. Roger Porter); and 40 are patients of the Hypertension-Endocrine Branch, NHLBI, screened for absence of psychiatric or neurological disease. Collection of data is now complete and analysis is under way. At the present time, the major hypothesis of the study appears to be confirmed. Affectively ill and epileptic patients were both found to have elevated incidence of transient visual, auditory, and olfactory changes, including both illusions and hallucinations. Epileptic, but not affective patients had elevated frequency of gustatory, visceral, and tactile sensory phenomena, and of involuntary motor symptomatology. Affectively ill but not epileptic subjects were distinguished by presence of cognitive illusions and distortions involving time.

Proposed course:

The relationship between sensory and cognitive phenomena and parameters related to course of illness, personality type, and electroencephalogram are now being analyzed. Plans will be formulated for follow-up studies relating presence or absence of these symptoms to biologic factors and treatment response.

2. Perception and recall of emotional and neutral stimuli in depression.

It is a common clinical observation that affectively ill patients often seem highly insensitive to their own internal emotional state. It is also well-known that depressed subjects perform more poorly than controls in a variety

of memory tasks. The purpose of this study is to look systematically at how depressed subjects evaluate the emotional qualities of verbal material, how such evaluations change with changes in clinical status, and how they interact with the subject's ability to recall the material. Fifteen depressed subjects were given a list of 40 words to rate for degree of emotionality on a zero-to-seven scale. Half the words were high emotion words, and half low emotion words, as determined by previous studies in normals. Subjects were asked to freely remember the words after they had been rated and later to pick out the words from a list in which they are intermixed with distractors. Data collection and analysis are complete at this point. A total of 31 depressed subjects and matched controls have been tested. The major findings of the study are as follows: Depressed and normal subjects do not differ in the way they rate emotionality of words. Similarly, both emotionality and concreteness of stimuli are robust memory aids for both groups. However, while depressed subjects are less benefited by both emotionality and concreteness in their free recall of words, they are more dependent upon both stimulus qualities for memory under recognition conditions. This pattern of results suggests that despite apparently similar evaluation of stimuli, the depressed process semantic aspects of material more shallowly than normals. This result is in accord with evidence in the literature that depressed are impaired primarily in tasks demanding deep or effortful processing of material. A manuscript has been submitted for publication.

Proposed course:

Studies are now being planned to investigate in greater detail the nature of the processing deficit in depression. In particular, the question of whether these patients are incapable of deep processing, or whether deep processing is decoupled from storage in memory will be explored.

3. Hypothesis testing in depression.

Most cognitive research in depression has focused on memory-related impairments. The present investigation uses a learning paradigm which places relatively light memory demands on the subject, focusing instead on hypothesis formulation and testing. The task, devised by Levine, is a variation on the Wisconsin Card Sort. Subjects are presented with the Levine task, which is then repeated with our own modifications. The task involves a deck of cards with two stimuli on each card. Each stimulus has four attributes (such as color and size). The subject's task is to guess which attribute has been arbitrarily designated as "correct." Subjects are asked to point to the stimulus that they think has the chosen attribute and are given yes or no feedback on selected cards. The procedure is devised so that each administration can be scored for number of hypotheses formed, number of correct hypotheses, ability of the subject to narrow down his choices as more feedback is given, and degree of hypothesis changing or keeping after positive or negative feedback. In addition, our modification of the procedure allows discrimination between poor performance due to memory deficits, or to subjects' inability to formulate an appropriate strategy. The collection and analysis of data are now complete. Depressed subjects were found to perform more poorly on the task than controls. Two components of performance distinguished depressed and control subjects, and also accounted for significant proportions of the variance in depressive performance.

These were poor "focusing," or inability to efficiently narrow down the list of possible solutions to the problem, and perseveration on hypotheses which have been disconfirmed. Such a pattern of performance bears similarities to patients with both Korsakoff's dementia and right temporal lobe lesions. While the analysis suggested that, at an elementary level, logic, memory, and attention were intact in the depressed patients, the apparent inability to coordinate these functions in a complex mental task played an important role in the depressive deficit. A manuscript has been submitted for publication.

Proposed course:

Studies will be initiated comparing abstract reasoning and memory performance on the same patients. Studies of these parameters in depressed-recovered patients are also being planned.

4. Lateralized hemispheric function in depression.

This study involves lateralized tachistoscopic presentation of visual material to depressed and control subjects. The task involves the subject making "same" and "different" judgments on material that can be processed either linguistically (left hemisphere) or on the basis of form (right hemisphere). Data have been collected and analyzed on ten female depressed patients, nine normal female controls, and nine normal male controls. Normal subjects showed the expected right visual field (left hemisphere) advantage in reaction time on the obligatory linguistic task, and little lateralization on the portion of the task which could be processed either verbally or spatially. By contrast, depressed subjects showed overall left visual field (right hemisphere) advantage, which was mostly attributable to the verbal portion of the task. Thus, the results appear to represent a shift in the location of functions as they are usually performed in normal subjects, rather than merely a change in level of activation or efficiency of the hemispheres. Such a result is congruent with a variety of studies in the literature suggesting a shift in hemispheric activity away from the left and toward the right hemisphere in depression. A manuscript detailing these results has been submitted for publication.

Proposed course:

Further laterality studies will attempt to look at changes in male depressed subjects, and to collect data in recovered depressed patients.

5. Relationship of cognitive deficit to diagnostic subtype and neuroendocrine changes in depression.

The purposes of this study are to attempt to examine cognitive dysfunction in depression as a function of diagnostic subtype. The study is being run in collaboration with the Evaluation Unit at the Psychiatric Institute, Washington, D.C., headed by Dr. Steven Targum. Classification procedures for depressed subjects include complete DSM III diagnosis, as well as biological indices provided by the dexamethasone suppression test and the TSH response to thyrotropin releasing hormone (TRH). Cognitive testing was designed to examine not only level of performance but the structure of deficits. A battery of six

memory tests were used for this study. The tests assessed the effect of type of processing (deep vs. shallow), type of recall (free vs. cued), type of stimuli (high vs. low emotional, high vs. low imageable), and level of organization of the stimuli. Level and structure of cognitive performance were examined as a function of diagnosis and also in relation to underlying metabolic abnormalities. A total of 27 depressed patients and 16 matched controls have been tested. As a whole, the depressed patients tended to perform below the level of normal controls. Depressed subjects were dichotomized according to normal vs. abnormal response to dexamethasone and thyrotropin releasing hormone (TRH) and according to presence or absence of DSM III diagnosis of Melancholia. The three methods of dichotomization proved independent in this sample. While cognitive performance did not differ according to presence or absence of Melancholia, or response to TRH, dexamethasone response did have implications for cognitive function. Those who failed to normally suppress cortisol following dexamethasone (escapers) were indistinguishable from normals in their memory performance, while dexamethasone suppressors showed the typical depressive deficit. Dexamethasone escapers were also distinguished from suppressors on a number of structural parameters relating to memory. Escapers and suppressors did not differ in age or level of education, or on measures of depression, anxiety, hopelessness, or atypicality. That a biologically abnormal subgroup of depressives may be intact cognitively is an unexpected and challenging finding. Two explanations are that (1) hypothalamic-pituitary hyperactivity in depression has an enhancing effect on memory, or (2) the metabolic deficit in dexamethasone escapers is a mechanism of depression which bypasses cognitive functioning, while other types of depression may be more cognitively related. Some evidence for the latter hypothesis was provided by the observation that ratings on the Beck Hopelessness Scale correlated significantly with memory performance in the suppressor but not the escaper group.

Proposed course:

Additional data are now being collected to attempt to corroborate these results and to expand the battery of cognitive tasks. A paper describing these findings was presented at the annual meeting of the American Psychiatric Association, May 1982.

Significance to biomedical research and to the program of the Institute:

These investigations are a part of the program of basic research at NIMH arrived at elucidating the nature of affective illness. Cognitively related studies are relevant to this goal from three points of view: (1) they concern an important area of deficit in affective illness, (2) they define an aspect of dysfunction which may provide clues to the pathologic anatomy and physiology of affective illness, and (3) they may provide useful information relating to clinically meaningful classification of affective disorders.

Publications:

None

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|---|--|--|-----|---------------------|--------------------|-----|------|--------|----------------|---------------------------------|-----|------|--|-----------------|--|-----|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00502-03 LPP | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Atypicality in Major Depressive Illness | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Edward K. Silberman</td> <td style="width: 30%;">Clinical Associate</td> <td style="width: 10%;">LPP</td> <td style="width: 10%;">NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Robert M. Post</td> <td>Chief, Section on Psychobiology</td> <td>BPB</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Frank W. Putnam</td> <td>Staff Psychiatrist, Section on Psychobiology</td> <td>BPB</td> <td>NIMH</td> </tr> </table> | | | PI: | Edward K. Silberman | Clinical Associate | LPP | NIMH | OTHER: | Robert M. Post | Chief, Section on Psychobiology | BPB | NIMH | | Frank W. Putnam | Staff Psychiatrist, Section on Psychobiology | BPB | NIMH |
| PI: | Edward K. Silberman | Clinical Associate | LPP | NIMH | | | | | | | | | | | | | |
| OTHER: | Robert M. Post | Chief, Section on Psychobiology | BPB | NIMH | | | | | | | | | | | | | |
| | Frank W. Putnam | Staff Psychiatrist, Section on Psychobiology | BPB | NIMH | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Unit on Psychobiology, BPB, NIMH | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.5 | PROFESSIONAL: 1.0 | OTHER: 0.5 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | |
| <p> To explore the possibility of an important <u>atypicality dimension</u> within the group of <u>primary, endogenous depression</u>, we have constructed a <u>rating scale</u> for atypical depressive illness. Forty-four NIMH patients were rated, all meeting Research Diagnostic Criteria for primary, major depressive illness. Atypicality in this group was characterized by <u>lack of encapsulated episodes</u>, <u>interpersonal difficulties</u>, evidence of <u>narcissistic character disorder</u>, and <u>high anxiety</u>, and <u>somatization</u>. Though atypical patients were <u>younger</u> than typical, they were <u>hospitalized significantly more often</u>. Biologically, they had smaller variance in two measures of <u>norepinephrine metabolism</u>, as well as <u>lower</u> levels of <u>platelet MAO</u>. Typicals were significantly more likely to have an <u>antidepressant response to sleep deprivation</u> than atypicals. Thus, atypicality as a dimension within primary, major depression may have important theoretical and clinical implications. </p> | | | | | | | | | | | | | | | | | |

Project Description

The initiation of this project was based on our observation of considerable heterogeneity of clinical presentation within the group of patients meeting Research Diagnostic Criteria for primary, endogenous, major depressive disorder. We reasoned that this clinical diversity may contribute significantly to the biological and prognostic heterogeneity within this group. To investigate the possibility of important subtypes or of an important atypicality dimension within this group, we have constructed a rating scale for atypical depressive illness. The scale is designed to measure degree and type of divergence from a classical syndrome of discrete episodes of autonomous mood and vegetative symptoms.

Forty-four NIMH patients were rated, all meeting Research Diagnostic Criteria for primary, major depressive disorder. In addition, 91% of these met criteria for endogenous depression as well. There were 21 men and 23 women; 28 patients were bipolar and 16 unipolar. In a preliminary session, global atypicality ratings on a zero to ten scale were derived by consensus of trained raters from the psychiatry, social work, and nursing services. Subsequently, the same group consensually rated patients on each of 24 items, to give a total scale atypicality score. Scale items assessed degree of autonomy of symptoms, vegetative signs, personality disorder, neurotic symptoms, thinking and perceptual disturbances, past course of illness, and premorbid adjustment.

Atypicality ratings derived by the two different methods were highly correlated ($r = .83$, $p < .0001$). Atypicality was characterized by relative lack of encapsulated episodes, disturbed interpersonal relationships, narcissistic entitlement, manipulateness, and prominent anxiety and somatization. Atypicality scores were not related to severity of depression. Atypical patients were significantly younger than typical patients ($p < .002$). Bipolar patients were significantly more typical than either bipolar II or unipolar patients ($p < .05$); however, the difference no longer reached significance when age was covaried out. Atypical patients were hospitalized significantly more often ($p < .02$) than typical patients, even though the typicals tended to be faster cyclers than the atypical. Biologically, the typical group had significantly less variance in measures of MHPG metabolism as reflected in urinary MHPG ($p < .05$) and spinal fluid norepinephrine ($p < .03$). While the atypicals tended to have higher MHPG levels, they had lower platelet MAO ($p < .056$). Typicals were significantly more likely than atypicals to have an antidepressant response to sleep deprivation than atypicals ($p < .1$).

The preliminary data cited above suggest that atypicality may be an important factor within primary, endogenous depression. It is not yet clear whether atypicality is best conceptualized as a subgroup or as a dimension within depression. Because in a research setting, we are dealing with a selected population, the interpretation of lower age and more frequent hospitalizations in the younger group is not yet clear. On the other hand, it may be that depression presents in a more varied and atypical manner when the patient is young, but evolves into the classical syndrome later in the patient's course. On the other hand, it may be that the atypical group is distinct from the typicals, with a greater propensity for regressive episodes and hospitalization. It is notable that within our group, atypicality was represented by features of narcissistic character disorder and disturbed interpersonal relationships, rather than by

schizoid trends or thinking disorders. Again, this may be a result of screening that would not apply to the general depressive population. There was an indication that the bipolar I group was highly typical, while the bipolar II and unipolar patients were equal in atypicality. Because age and polarity were confounded in our patient population, it was impossible to test reliably for an association between typicality and polarity.

Biologically, the trend toward higher MHPG and lower variance in norepinephrine metabolism can be placed in the context of previous biological studies. Generally speaking, lower MHPG has been associated with more classical and more severe forms of depression. While low platelet MAO has also been associated with more classical (bipolar) depression, it is also associated with a group of people within the general population who have high levels of personality disorder, particularly sensation-seeking and sociopathy. Thus, within our group, atypical patients tended to have low MAO, while typical patients had either high or low MAO.

Significance to biomedical research and to the program of the Institute:

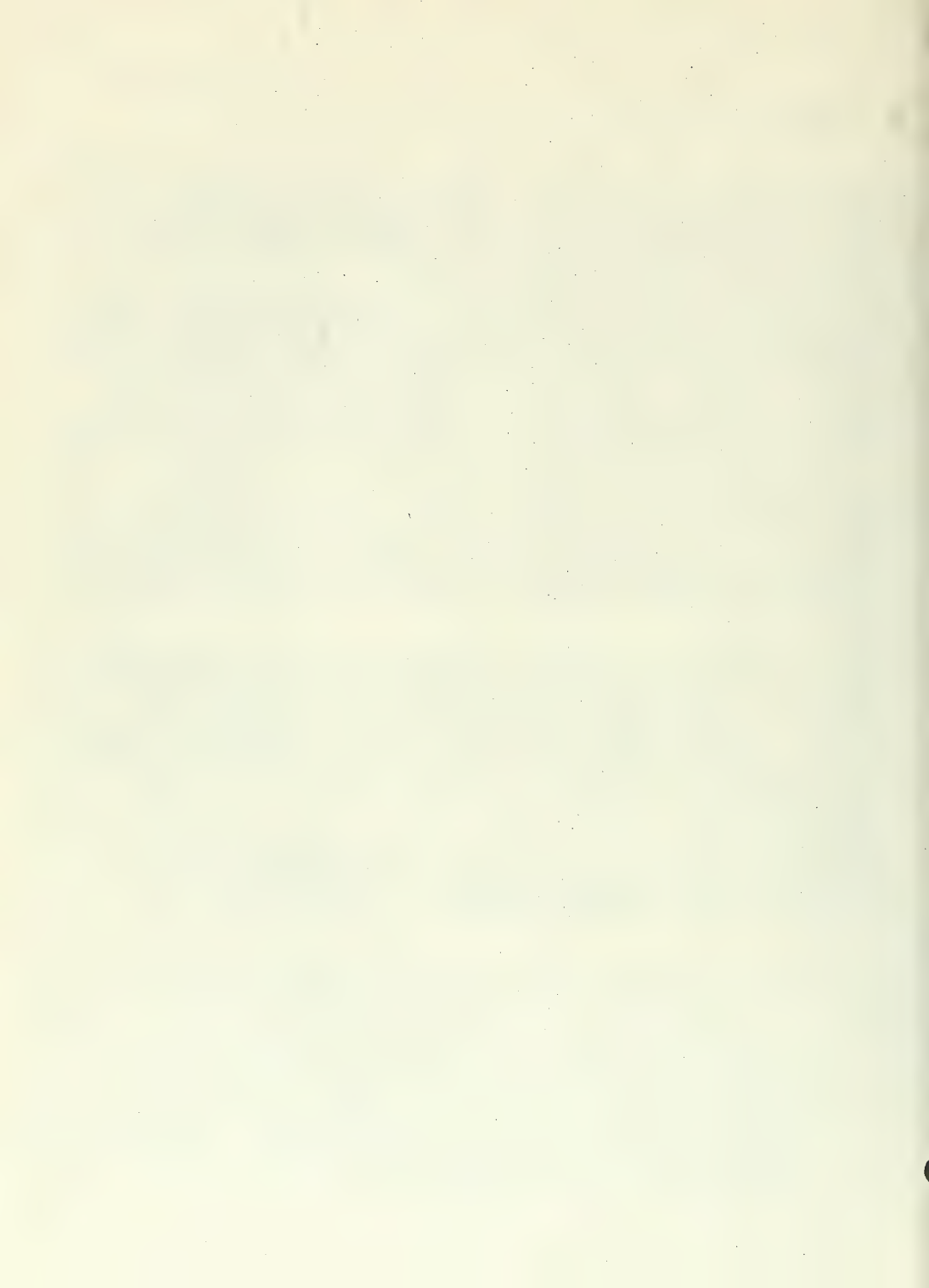
This project falls within the context of studies designed to relate biologic factors (including dry response) to clinical variables. It is thus a facet of the Institute's ongoing attempt to define the behavioral implications of "biological markers," and to further refine classification procedures in affective illness.

Proposed course:

While the above data were gathered retrospectively by consensus, plans are now under way to rate subjects as they enter the hospital. Working with a fresh, common data base will allow higher reliability than retrospective consensus ratings. Information gathered in this way will enable us to examine correlates of varying types of atypicality, rather than just total scale scores. In addition, we are collecting data on course of illness and response to treatment after NIH hospitalization.

Publications:

Silberman, E.K., Post, R.M.: Atypicality in primary depressive illness: A preliminary survey. Biological Psychiatry 17: 285-304, 1982.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00503-02 LPP |
| PERIOD COVERED <u>October 1, 1981 to September 30, 1982</u> | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Human Clinical Studies of Attention Disorders</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Allan F. Mirsky OTHER: Connie C. Duncan-Johnson Richard Coppola Herbert Weingartner Theodore P. Zahn Richard Nakamura Roger Porter Judy Rumsey Fritz Dreifuss | Chief Senior Staff Fellow Engineer Officer Chief, Unit on Cognitive Studies Research Psychologist Senior Staff Fellow Chief Staff Fellow Professor of Neurology | LPP NIMH LPP NIMH LPP NIMH LPP NIMH LPP NIMH LPP NIMH EBB NINCDS BPB NIMH University of Virginia |
| COOPERATING UNITS (if any) Epilepsy Branch, Clinical Neurosciences Branch, NINCDS; Biological Psychiatry Branch, NIMH; University of Virginia | | |
| LAB/BRANCH <u>Laboratory of Psychology and Psychopathology</u> | | |
| SECTION | | |
| INSTITUTE AND LOCATION <u>NIMH, ADAMHA Bethesda, Maryland 20205</u> | | |
| TOTAL MANYEARS: <u>3.0</u> | PROFESSIONAL: <u>1.75</u> | OTHER: <u>1.25</u> |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> This research comprises three related areas of investigation concerned with specifying <u>neuropsychological</u> factors underlying clinical conditions in humans in which <u>disturbed attention</u> is a major symptom. A major emphasis is on (1) illuminating the nature of brain stem pathophysiology, if any, in such entities as petit mal or <u>absence epilepsy</u>, <u>infantile autism</u>, <u>schizophrenia</u>, and related diseases; (2) an additional major emphasis is on extending the neuro- behavioral analysis of attention loss in absence epilepsy so as to facilitate developing alternative treatment strategies for such patients. Both of these projects form part of a larger effort which is aimed at (3) developing a comprehensive and systematic <u>taxonomy of attentional disorders</u> in humans. This latter study will eventually comprise study of patients with <u>cerebral lesions</u>, <u>seizures</u>, <u>dementing diseases</u>, and <u>metabolic illnesses</u> of the brain. </p> | | |

Project Description:

1. Brain stem mechanisms in attention impairment.

Current approaches to the neuropsychology of attention impairment have emphasized that the system responsible for the maintenance of attention or consciousness within the brain is most likely represented at a variety of levels of the neuraxis. From an evolutionary point of view, it is clear that the capacity for sustained attentive behavior is present in many species which do not possess more than a rudimentary forebrain or telencephalon. MacLean's analysis of the R-complex within the human brain leads to the view that this "clump of ganglia," which constitutes virtually all of the reptilian brain, can support a variety of ritualistic, repetitive behaviors which could be characterized as sustained and attentive. Evolution progressed and the brain developed additional complexity and volume. Additional capacity for attentive behavior was thus overlaid on the more primitive, although in many aspects thoroughly adequate, brain stem system of the reptile. Therefore, although the system for maintenance of attentive behavior in the human (or higher primate) includes limbic and neocortical components, the brain stem remains a key component and possibly the keystone of the entire system. Authors such as Hughlings Jackson and Penfield and Jasper recognized this in their conceptions, respectively, of "highest level seizures" and the "centrencephalon." In their theorizing, consciousness was either localized in or regulated by deep brain stem structures. Without reviewing all of the evidence that led to those views of the hierarchical organization of attention and consciousness within the brain, we nevertheless point to the extremely deleterious effects on such capacities of small lesions in the brain stem region of the third and fourth ventricles. In the last ten years, a new technological refinement of evoked potential methodology has made possible an other-than-theoretical exploration of the role of brain stem structures in certain clinical states. This "far field" technique makes it possible to assess the integrity of auditory (and somatosensory) relay nuclei within the brain stem of humans. Although the technique has probably had most utilization in the diagnosis of demyelinating disease, it has also been used in the study of other neurological and, recently, psychiatric disorders. There may or may not be any specific interest in these sensory systems (auditory, somatosensory) in studying a particular clinical entity (i.e., absence seizures, infantile autism); nevertheless, the possibility of evaluating the functional integrity of certain systems within the brain stem is extraordinarily valuable, and many clinical investigators are using these techniques. We have published work indicating that there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in infantile autism. We have also shown that in absence seizures (spike-wave activity), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brain stem functioning. We are planning to continue such studies with these patient groups and others once our facilities have been developed in the ACRF.

2. Neurobehavioral studies in absence epilepsy.

We have for a number of years been studying the absence attack in patients with petit mal/centrencephalic/absence seizures (the terms are more or less interchangeable) as model state to understand the phenomenon of consciousness/

attention. Some of these studies have involved comparing the behavioral capacities of patients suffering from petit mal--as opposed to focal seizure disorders; other studies have involved detailed comparison and contrast between the behavioral and the electroencephalographic symptoms/signs of the disorder. Most recently these investigations have: (1) used evoked potentials in the visual and auditory modalities as indices of the sensory effects of generalized seizure activity of the symmetrical and synchronous spike and wave (SW) variety, and (2) examined changes in the EEG power spectrum prior to SW bursts as prodromal signs which may be used to predict (and ultimately to control) SW bursts. We propose to continue this line of neurobehavioral investigation, using event related potentials of various types as well as other behavioral and physiological tools, to refine further our understanding of the nature of altered consciousness in absence (petit mal) epilepsy.

3. A taxonomy of attentional disorders.

The goal of this project is to develop a comprehensive and coherent account of the relation between symptoms of altered or disturbed attention or consciousness as they appear in various clinical entities, the other behavioral and clinical characteristics of the several disorders, and the specific central nervous system damage or disturbance in each disorder. The attentive capacities of the patients will be assessed by a number of measures comprised within the GAT (generalized attention test) which is an outgrowth of the CPT (continuous performance test) a measure of sustained visual attentive behavior. The ultimate goal will describe the precise attentive deficit (as opposed to cognitive losses) and the nature of the neuropathophysiology associated with each of the following clinical entities:

- cerebral cortical lesions (frontal, parietal, or temporal lobe)
- centrencephalic/absence epilepsy
- schizophrenia
- infantile autism
- dementing diseases (Alzheimer's, Korsakoff's, Huntington's).

We will attempt, as well, to relate these changes where possible to standardized measures of mnemonic and other cognitive function, and to autonomic indices of attention, arousal, and habituation.

Significance to biomedical research and to the program of the Institute.

Since attention disturbance is a characteristic of many significant psycho- and neuropathological disorders, it is essential to have a clear empirical and theoretical account of the role and pathophysiological significance of the symptom. It will aid in understanding the etiology and course of these illnesses and may aid in improving their treatment.

Proposed Course:

We have run a small group of schizophrenic, epileptic, and brain-injured patients through our laboratory procedures (i.e., CPT, brain stem auditory evoked potentials, various tests of cognition and memory, autonomic indices of

attention, etc.). During the course of the next year, we hope to recruit additional cases from other diagnostic categories into this taxonomic study. However, since we have not had a laboratory to pursue this work, it has not been possible to achieve substantial progress during this reporting period.

Publications:

Siegel, A., Grady, C.L., and Mirsky, A.F. Prediction of Spike-Wave Bursts in Absence Epilepsy by EEG Power-Spectrum Signals. Epilepsia. 23:47-60, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00504-02 |
| PERIOD COVERED October 1, 1981 to September 1, 1982 | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Experimental models in the rhesus monkey of generalized seizures of the absence type.</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Allan F. Mirsky OTHER: Eva Bakay Pragay | Chief Research Psychologist | LPP NIMH LPP NIMH |
| | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA Bethesda, MD 20205 | | |
| TOTAL MANYEARS: 0.6 | PROFESSIONAL: 0.6 | OTHER: 0.0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>Generalized seizure activity with the electrographic appearance of <u>absence epilepsy</u> (bilaterally symmetrical and synchronous paroxysmal three-per-second spike and wave discharges) can be elicited in the <u>monkey</u> by a variety of methods. These include electrical <u>stimulation</u> of various locations within the brain, injection of <u>convulsant drugs</u> and other substances, and administration of compounds which may alter normal inhibitory mechanisms within the cell. Model seizure states created in these ways are studied in order to test hypotheses about pathophysiological seizure mechanisms, sensory processing and attentional capacities during absence seizures, effects of spike-wave activity on cellular activity, and effects of techniques or maneuvers which may modify or reduce convulsive activity. Most recently this project has involved the following work: we studied the (paradoxical) seizure inducing effects of a GABA-enhancer and the effects on auditory brain stem evoked potentials of generalized seizures induced by injection of pentylenetetrazol.</p> | | |

Project Description

γ -vinyl GABA and γ -acetylenic GABA are two recently synthesized compounds whose metabolic effects include the blocking of the enzyme action responsible for the metabolism of the inhibitory neuro-transmitter GABA. The accumulation of GABA thus produced should have an anticonvulsant action, and so it does, at moderate doses of these compounds. However, as the dose is increased, there is a paradoxical rebound effect and animals treated with large quantities of either γ -vinyl or γ -acetylenic GABA have shown paroxysmal seizure activity. And of interest to us is the fact that the seizure activity is not the clinically obvious generalized tonic-clonic variety. Instead, although widespread spikes and spike-wave patterns may be seen, there may be few clinical signs. Such an effect is reminiscent of absence seizures (staring spells) in human centrencephalic epilepsy. We are in the process of exploring the utility of these compounds for producing model seizures of the petit mal variety in the monkey.

We have also induced generalized seizure activity in rhesus monkeys, reflected in both clinical and EEG manifestations, by systemic administration of pentylenetetrazol. Brain stem auditory evoked potentials (BAEP) were recorded from indwelling epidural electrodes at the vertex of the skull as well as from electrodes implanted along the primary auditory pathway in the brain stem (inferior colliculus). Several components of the complex "far field" vertex potential showed increased latency and decreased amplitude during ictal episodes as compared to control periods both pre-drug and post-seizure. Similar changes were seen in direct recordings from brain stem auditory structures. The parallel recording of "far field" (vertex) potentials and "near field" (brain stem auditory pathway) potentials appears to be a fruitful approach. The direct recording from brain stem auditory structures adds reliability and temporal resolution to the findings. Thus, in contrast to the vertex potential which requires several hundreds, or even thousands of stimulus repetitions, only a few samples are necessary to obtain reliable waveforms from direct brain stem recordings. Consequently, the grain and the resolution of the experiment can be enhanced, and various periods of pre-, during- and post-ictal stages as well as phases of gradual recovery can be assessed. The analysis of small consecutive samples revealed profound BAEP changes not only during the ictal period but immediately following the seizure activity. There was also marked fluctuation of suppression and potentiation during the post-ictal recovery period.

Significance to biomedical research and to the program of the Institute:

This experiment provides direct evidence of brain stem involvement in consciousness and in generalized seizures and contributes to the current efforts to produce an accurate primate-based model of the pathophysiological processes in absence epilepsy.

Proposed course:

We will be continuing with this experimental program as primate facilities become available to LPP. We can report no progress on this project during the past year since we had no laboratory available to pursue this work.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00505-02 LPP | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | |
| TITLE OF PROJECT (80 characters or less) Brain Lesion and State Change Effects on Visual Attention | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 40%; vertical-align: top;"> PI: Richard K. Nakamura OTHER: Allan F. Mirsky Richard Coppola Eva Bakay Pragay Mortimer Mishkin H. Enger Rosvold David Friedman Louis Sokoloff Charles Kennedy Masanori Ito J. Christian Gillin Richard Wyatt William Freed John Morihisa </td> <td style="width: 40%; vertical-align: top;"> Senior Staff Fellow Chief Engineer Officer Research Psychologist Acting Chief Research Physiologist Senior Staff Fellow Chief Guest Worker Fogarty Scholar Research Psychiatrist Director Senior Staff Fellow Clinical Associate </td> <td style="width: 20%; vertical-align: top;"> LPP NIMH LPP NIMH LPP NIMH LPP NIMH LN NIMH LN NIMH LN NIMH LCM NIMH LCM NIMH LCM NIMH BPB NIMH SMRAP NIMH SMRAP NIMH SMRAP NIMH </td> </tr> </table> | | | PI: Richard K. Nakamura OTHER: Allan F. Mirsky Richard Coppola Eva Bakay Pragay Mortimer Mishkin H. Enger Rosvold David Friedman Louis Sokoloff Charles Kennedy Masanori Ito J. Christian Gillin Richard Wyatt William Freed John Morihisa | Senior Staff Fellow Chief Engineer Officer Research Psychologist Acting Chief Research Physiologist Senior Staff Fellow Chief Guest Worker Fogarty Scholar Research Psychiatrist Director Senior Staff Fellow Clinical Associate | LPP NIMH LPP NIMH LPP NIMH LPP NIMH LN NIMH LN NIMH LN NIMH LCM NIMH LCM NIMH LCM NIMH BPB NIMH SMRAP NIMH SMRAP NIMH SMRAP NIMH |
| PI: Richard K. Nakamura OTHER: Allan F. Mirsky Richard Coppola Eva Bakay Pragay Mortimer Mishkin H. Enger Rosvold David Friedman Louis Sokoloff Charles Kennedy Masanori Ito J. Christian Gillin Richard Wyatt William Freed John Morihisa | Senior Staff Fellow Chief Engineer Officer Research Psychologist Acting Chief Research Physiologist Senior Staff Fellow Chief Guest Worker Fogarty Scholar Research Psychiatrist Director Senior Staff Fellow Clinical Associate | LPP NIMH LPP NIMH LPP NIMH LPP NIMH LN NIMH LN NIMH LN NIMH LCM NIMH LCM NIMH LCM NIMH BPB NIMH SMRAP NIMH SMRAP NIMH SMRAP NIMH | | | |
| COOPERATING UNITS (if any) Laboratory of Neuropsychology, Laboratory of Cerebral Metabolism, Biological Psychiatry Branch, Special Mental Research Adult Psychiatry Branch | | | | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | | | | |
| SECTION | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | | | | |
| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 2.0 | OTHER: 1.0 | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | |
| <p>This project consists of four related areas of investigation, all concerned with the analysis of mechanisms of <u>attention</u>. Special emphasis is placed on the use of <u>lesion and/or biochemical techniques</u> to elucidate mechanisms involved in visual attention. The four areas are: (1) attention and cerebral mechanisms of visual behavior; (2) pharmacological mechanisms of attention; (3) brain activity in inattention: <u>local cerebral glucose metabolism in sleep</u>; and (4) the design of a general attention task.</p> | | | | | |

Project Description:

1. ATTENTION MECHANISMS AND VISUAL BEHAVIOR:

We have found that large nonvisual lesions of cerebral cortex in the monkey cause permanent blindness. The basic preparation is as follows: one hemisphere is visually deafferented by combined optic tract transection and forebrain commissurotomy. The other hemisphere has all cortical areas removed except for the striate, prestriate and inferior temporal visual areas. Animals prepared in this way were functionally blind for over a year despite anatomical evidence of an intact visual system. We have been analyzing this phenomenon in an effort to determine the role of nonvisual cortex in visual behavior and to establish the nature of higher processing in the visual system. The major results indicate that we are beginning to elucidate the role of attention in sensory processing.

We have examined the single neuron responses to visual stimuli in the visual systems of our blind monkeys. Results of over 400 neurons studied to date in the blind animals, compared to over 200 neurons in normal animals, indicate that visual responses of the blind are near normal in both striate and prestriate areas. Preliminary data from inferior temporal cortex, on the other hand, suggest that the blind animals show less specificity of neuronal response than seen in the normals. These data imply that in the absence of feedback from higher cortical structures and in the absence of behavioral feedback, the visual system continues near normal processing through to the prestriate cortex. Only at the level of inferior temporal cortex does the effect of such losses of feedback change cellular responses to visual stimulation. This in turn suggests that the effects of attention on neuronal responses will not be significant in areas earlier in the visual pathway than inferior temporal cortex.

In an effort to determine the critical cortical zones of this blindness phenomenon, the large nonvisual ablation has been subdivided into three parts: the sensorimotor cortex, the limbic cortex, and the polysensory cortex. These areas have been ablated in separate groups of monkeys, and the only lesion which produces a blindness effect is the polysensory cortical lesion. This area consists of dorsal prefrontal cortex, inferior parietal cortex, and superior temporal cortex (including insula). It is of considerable significance to us that a neglect or inattention syndrome follows the ablation of any portion of the polysensory area, for this suggests that our animals cannot see because they cannot attend to the visual modality.

The absence of effect of the sensorimotor lesion creates confidence that the blindness is not simply the result of a disconnection of visual input from motor output. In the original chronic blindness preparation, the non-visual lesion is placed in one hemisphere, the optic tract to the other is cut and the forebrain commissures are divided. If, however, the forebrain commissures are left intact, then there will be a period of blindness lasting approximately 10 to 40 days. This period is followed by a recovery of visual function which permits not only visual guidance to food but discrimination of visual patterns as well.

This recovery of visual function, when a path of communication is left between the hemispheres, indicates that the blindness is the result of a disconnection of

vision from higher cortical processing areas. Combined with earlier results, we can be more specific and say that the blindness is caused by a disconnection of visual areas from polysensory (attentional) areas of the brain. Thus, a connection between visual and attentional areas appears to be critical before visual behavior can proceed in monkeys.

2. PHARMACOLOGIC MECHANISMS OF ATTENTION:

Striatal dopamine has been implicated as an important transmitter in the attention-arousal system. Areas A9 and A10 (substantia nigra pars compacta and the ventral tegmental area) have been shown to be major sources of dopamine for the brain in general and the cerebral cortex in particular. In rats, lesions of areas A9 and A10 have been associated with inattention or neglect, tremor, transient aphasia, and adypsia, and a deficit on the delayed alternation task. Little is known about the effects of such ablations in the monkey, though in man, cell loss and reduced dopamine in these areas have been associated with Parkinson's disease.

We have therefore begun a study of A9 and A10 lesions in monkeys. We are looking for deficits in attention, motor performance, delayed alternation, food and water intake, and reduced dopamine levels in caudate and frontal cortex. Results thus far indicate that lesioned monkeys show attention losses in the somatosensory and auditory modalities but not in vision. They also show little change in motor performance, food and water intake, and behavioral performance of delayed alternation or visual discrimination.

Because of our interest in striatal dopamine, we have begun a study with the division of Special Mental Health Research to investigate the possibility of transplanting dopamine-secreting tissue into the brains of monkeys that have been previously deprived of striatal dopamine with unilateral 6-hydroxydopamine ablations. Objectives of the research are to: (a) look at the effects of unilateral ablations of dopamine systems, (b) examine the practicability of brain tissue implants in the monkey, and (c) see if behavioral changes following dopamine ablations can be reversed with transplanted tissue. Preliminary data indicate that it is possible to transplant fetal substantia nigra tissue into a monkey brain and get both growth and dopamine secretions.

3. BRAIN ACTIVITY IN A STATE OF INATTENTION:

Major clues to the systems involved in attention might be derived from the study of natural states of reduced attention such as sleep. In collaboration with the Laboratory of Cerebral Metabolism, the Laboratory of Neuropsychology, and the Sleep Laboratory, we have been studying local cerebral metabolism and protein synthesis of monkeys in slow-wave sleep and wakefulness.

We have used the 2-deoxyglucose method to determine local cerebral metabolism in eight monkeys; four experimental animals in slow-wave sleep and four control animals that were kept awake. The major finding has been that the animals in sleep show an overall reduction in cerebral metabolism of about 30%. Further, we have been unable to find any brain structure which shows, on average, higher activity in slow wave sleep than in the awake state. Hypnogenic center theories,

which postulate a brain area which actively keeps an animal in sleep, are therefore not supported.

In addition, we have applied a new method to determine local cerebral protein incorporation to four monkeys; two in slow-wave sleep and two that were awake. Preliminary data from these animals show that protein synthesis, like cerebral glucose metabolism, is reduced throughout the brain in slow-wave sleep. Theories of sleep suggesting protein synthesis increases to make up for wear and tear during wakefulness are therefore contradicted.

Thus, in slow wave sleep, there appears to be a general diminution of brain activity. We will examine next the effect of paradoxical or REM sleep on brain activity. REM sleep is of particular interest because the organism is in a state of total inattention while at the same time the brain is electrographically very similar to the awake brain. Thus, the brain structures controlling the difference in attention stages may be more readily revealed.

4. DESIGN OF A GENERAL ATTENTION TASK:

While the foregoing studies may reveal important clues to the nature of attention, they all represent inherently indirect approaches because in none is a systematic attempt made to isolate the attentional process while controlling for other processes such as sensory input and motor output. If sensory input and motor output were held constant while attention was made to switch from one aspect of the sensory input to another, then it would be possible to study directly the mechanisms of attention.

We have begun the process of designing a general purpose test which will allow such a direct examination of attention. Included are additional design features such as: (a) the parameters of the task will be usable for both humans and monkeys; (b) it will accommodate both electrophysiological and behavioral analysis by permitting quantification of attention changes and allowing precise timing of brain events associated with these changes; and c) information will be transmitted through the visual system to take advantage of the considerable information available on visual processing.

Proposed course:

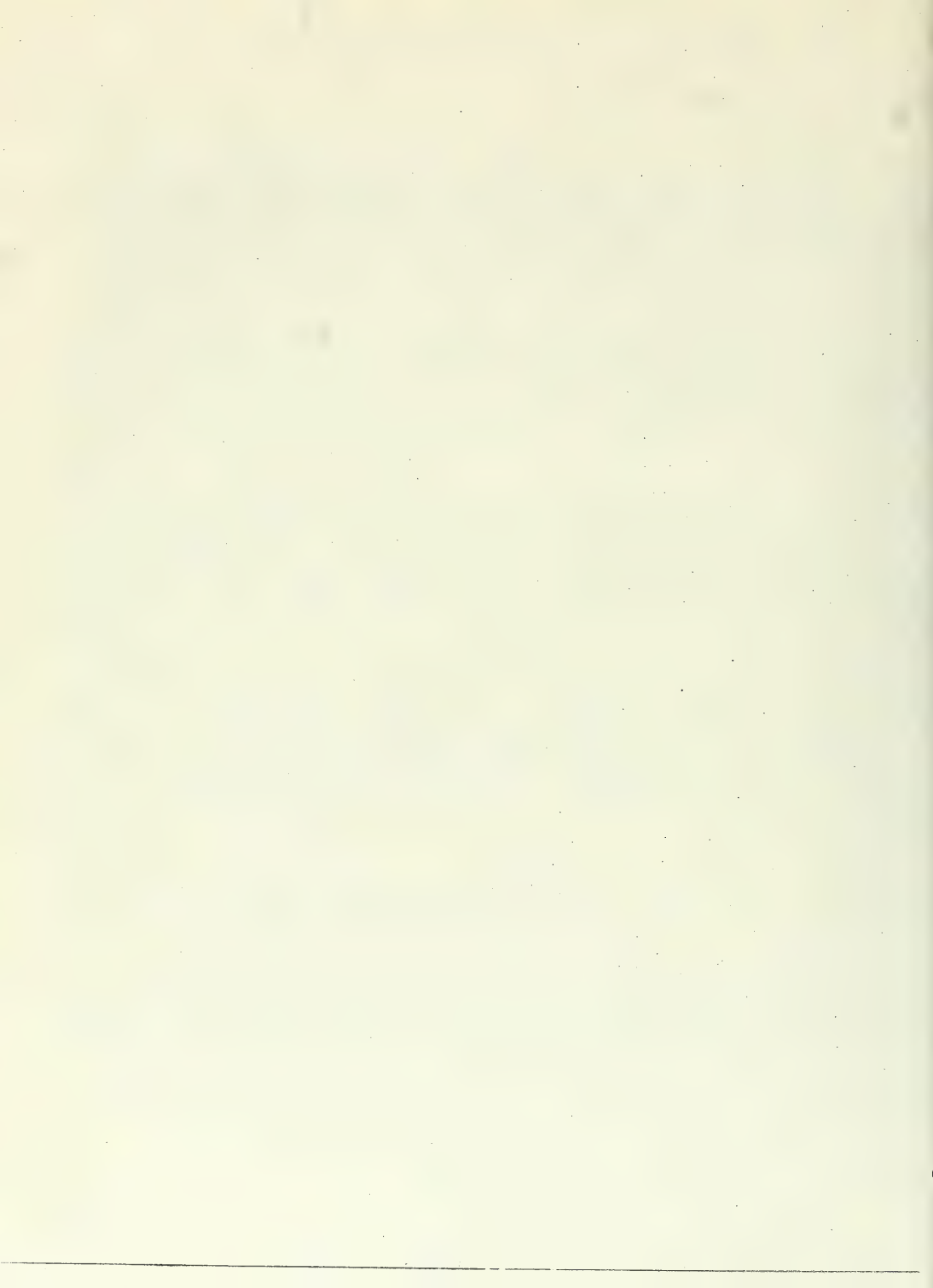
Implementation of this task has begun and feasibility studies will be performed this year on both monkeys and humans.

Significance:

We hope to understand the mechanisms underlying attention and consciousness in animals and man. This will enable us to develop adequate animal models of human clinical syndromes featuring reduction of attention or consciousness, such as schizophrenia, dementia, and petit mal epilepsy.

Publications:

Kennedy, C., Gillin, J.C., Mendelson, W., Suda, S., Miyaoka, M., Ito, M., Nakamura, R.K., Storch, F.I., Pettigrew, K., Mishkin, M., and Sokoloff, L.: Local cerebral glucose utilization in non-rapid eye movement sleep. Nature 297: 325-327, 1982.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 00506-02 LPP |
| PERIOD COVERED October 1, 1981 to September 1, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Attention-related neurons in the brain of the rhesus monkey | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Eva Bakay Pragay OTHERS: Allan F. Mirsky Ralph U. Esposito | Research Psychologist LPP NIMH Chief LPP NIMH Sr. Staff Fellow LPP NIMH | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Psychology and Psychopathology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 3.2 | PROFESSIONAL: 2.2 | OTHER: 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> This project is concerned with an analysis of the activity of nerve cells in that system within the <u>primate brain</u> which is necessary and responsible for the process we refer to as <u>attention</u>. Monkeys trained to perform <u>visually-guided go-no go discrimination tasks</u> are tested whilst <u>extra cellular recordings</u> are made from brain regions thought to be part of an attentional system. The most recent study in this series examined structures in the forebrain. </p> <p> We have found attention-related units in the anterior portion of the upper bank of the <u>cingulate sulcus</u>, in the rostral neostriatum, and in the periarculate area of the dorsal frontal cortex. These cells responded to the manipulation of attention, e.g., manipulation of the pre-stimulus waiting period or changing the behavioral significance of the task-stimuli. These attention-related units have properties similar to those found in the <u>brainstem reticular formation</u> in previous studies by Bakay Pragay, Mirsky, Ray, and colleagues. </p> | | |

Project Description:

In an effort to describe at a cellular level the brain system in the primate necessary for attentional behavior, we have developed a monkey preparation that permits such study. Extra-cellular recordings are made from trained animals while they perform on discrimination tasks requiring visual attention. In earlier work, we described "attention-related" cells in the mesopontine region of the brainstem. Later work has been aimed at forebrain structures, including the dorsolateral prefrontal cortex, the upper bank of the rostral portion of the cingulate sulcus, and the rostral neostriatum. More recently, this work was extended to more posterior aspects of the dorsolateral frontal cortex (including regions between the arcuate and central sulci), as well as to the corresponding regions around the cingulate sulcus.

As in prior studies, the task required the animal to press a "hold" button for 2 seconds in order to turn on a "cue" button; the latter was transilluminated by either a red ("go") or a green ("no go") cue-light. In the go trials, the animal had to release the hold button and press the cue button within 1 second. In the no go trials, it had to maintain pressure on the hold button for another second. In the basic task, both correct go and no go trials were rewarded.

The task permitted us to distinguish response-related (Type I) and stimulus-related (Type II) cell types. Type I units, which responded only during go trials, can be regarded as related to the execution of the instrumental motor response. Type II neurons which respond during both go and no go trials could be related to various functions, which could be defined by their temporal relationship to various task-events (events in the trial), as well as by applying variations in the experimental conditions. Type II activity could be classified into the following subgroups: (1) post-stimulus, pre-reinforcement change; (2) anticipatory activity starting in the intertrial interval, usually in the form of a gradual increase in firing rate of the cell preceding stimulus onset ("pure" anticipation), or in addition, following stimulus onset (combined anticipatory and "evoked" activity); (3) pre-reinforcement activity. Post-stimulus (evoked) activity, whether in pure or combined form, could be symmetrical in shape and in magnitude for both go and no go trials, or asymmetrical, the latter occurring mostly in the form of the go-trial-related response being bigger (more intensive and/or more systematic and/or longer lasting) as compared to the no go trial-related response.

The attention-related property of the Type II units was tested by varying the within-task conditions. These included: (a) reward for both correct go and no go trials (basic task); (b) non-reinforcement for correct no go trials (NRNG); (c) varying the length of the fixed intertrial interval (ITI): 1 sec, 2 sec (basic task), and 3 sec (or 4 sec). In addition, two extra-task conditions were applied: visual stimuli (including the task-stimuli) without access to task or reward, and non-contingent delivery of reward. The NRNG condition and the extra-task administration of stimuli represented manipulation of attention through variation of the reward value of the stimuli. Variation of the length of the ITI manipulated attention by varying the possibility of development of a preparatory set.

The preliminary results indicate a difference in the distribution of various types of cells along the anterior-posterior dimensions of the forebrain areas described above. Units in the more anterior regions (in the periarculate area) plus in the corresponding levels around the cingulate sulcus showed the following characteristics: Type II was predominant over Type I; the Type II activity was predominantly anticipatory and or symmetrical for both go and no go trials. Type II units responded readily to the manipulation of attention, e.g., in terms of changing the length of their anticipatory activity as a function of the pre-stimulus waiting period (fore-period), or by changing the magnitude of their response as a function of stimulus significance. On the other hand, more caudal regions around the central sulcus and the corresponding pericingulate area showed predominantly Type I activity.

In addition to the anterior and posterior regions described above, an intermediate area (apparently at the junction zone of the premotor and the motor cortex) was also sampled. The cell population in this area showed "mixed" characteristics in various ways. It contained comparable amounts of Type II vs. Type I units. The majority of the Type II units showed combined anticipatory and evoked activity, as was the case of the Type II units in the anterior region. However, unlike the more rostral Type II units, their evoked activity was asymmetrical, the go-trial-related activity being more vigorous than the no-go-trial-related activity.

Concerning the functional significance of the various forms of Type II activity, the following propositions could be made: The "symmetrical" anticipatory units in the anterior forebrain may represent a preparatory sensory set which facilitates performance in an attention task. The functional significance of the asymmetric Type II cells - found mainly in the post-arcuate - pre-central area is less clear. They may be considered visuo-kinetic, as suggested by Kubota and colleagues, and thus, comparable to the combined signal plus movement units identified recently by Wise and Weinrich in the premotor cortex. At any rate, these asymmetric Type II cells seem to represent a later (possibly transitional) stage in information processing as compared to the pure symmetrical-anticipatory activity.

Significance to biomedical research and to the program of the Institute:

This study is an important step forward in our program of attention research. It represents a second step in the line of exploring various brain regions, by means of extra-cellular unit recording techniques in a go no-go task. Any final assignment of function must, of course, await histological confirmation of the electrode locations. However, this work provides an important basic research underpinning for our clinical studies of attention disorders.

Proposed course:

We hope to add additional data from other animals that will be studied over the next two years.

Publications:

Mirsky, A.F. and Bakay Pragay, E. Brain mechanisms in the processing of sensory information: Clinical symptoms, animal models, and unit analysis. In: D.E. Sheer (Ed.), Attention: Theory, brain functions, and clinical application. Hillsdale, N.J.: Erlbaum, in press.

Ray, C.L., Mirsky, A.F., and Bakay Pragay, E. Functional analysis of attention-related unit activity in the reticular formation of the monkey. Experimental Neurology, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00671-13 LSES | | | | | | |
| PERIOD COVERED <u>October 1, 1981, to September 30, 1982</u> | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Social Origins of Stress</p> | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Leonard I. Pearlin</td> <td style="width: 33%;">Research Sociologist</td> <td style="width: 33%;">LSES NIMH</td> </tr> <tr> <td>Other: Clarice Radabaugh</td> <td>Social Science Technician</td> <td>LSES NIMH</td> </tr> </table> | | | PI: Leonard I. Pearlin | Research Sociologist | LSES NIMH | Other: Clarice Radabaugh | Social Science Technician | LSES NIMH |
| PI: Leonard I. Pearlin | Research Sociologist | LSES NIMH | | | | | | |
| Other: Clarice Radabaugh | Social Science Technician | LSES NIMH | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">None</p> | | | | | | | | |
| LAB/BRANCH <u>Laboratory of Socio-environmental Studies</u> | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION <u>NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</u> | | | | | | | | |
| TOTAL MANYEARS: <p style="text-align: center;">0</p> | PROFESSIONAL: <p style="text-align: center;">0</p> | OTHER: | | | | | | |
| CHECK APPROPRIATE BOX(ES) | | | | | | | | |
| <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The principal investigator retired from Government service at the end of Fiscal Year 1981 and transferred the project to the University of California, where he is now employed. | | | | | | | | |

Publications:

Pearlin, L.I., Lieberman, M.A., Menaghan, E.G., and Mullan, J.T.:
The Stress Process. J. Health Soc. Behav. 22: 337-356, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00672-17 LSES |
| PERIOD COVERED <u>October 1, 1981, to September 30, 1982</u> | | |
| TITLE OF PROJECT (80 characters or less) Social Psychological Correlates of Occupational Position | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: M. L. Kohn Other: C. Schooler J. Miller K. Miller K. Slomczynski W. Wesolowski W. FitzGerald K. Tominaga C. Schoenbach | Chief, Lab of Socio-environmental Studies Research Psychologist Research Sociologist Research Sociologist Visiting Scientist Visiting Scientist Research Sociologist Visiting Scientist Social Science Analyst | LSES NIMH LSES NIMH LSES NIMH LSES NIMH LSES NIMH LSES NIMH LSES NIMH LSES NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Socio-environmental Studies | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 11.5 | PROFESSIONAL: 6.5 | OTHER: 5 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The object of this study is to assess the <u>reciprocal effects of occupational conditions and psychological functioning</u> (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a <u>longitudinal study</u> in 1974, with the reinterviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children. <u>Replications</u> of this research have been carried out in <u>Poland</u> and <u>Japan</u> . | | |

Project Description:

The principal goal of this research is to assess the relationships between people's job conditions and their psychological functioning. The evidence provided by this research demonstrates that job conditions have a marked impact on cognitive functioning, on values, and on conceptions of self and orientations to society.

The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study has been to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators have attempted to assess the magnitudes of the reciprocal effects of job conditions and several important facets of psychological functioning. The study of wives was designed to ascertain whether job conditions affect men and women similarly. The research has shown that they do. These data should also enable the investigators to assess the effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning, in each case taking account of the individual's own job experiences. The study of the children was designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, the major research efforts have been addressed to five objectives: (1) Using the longitudinal data for men, reanalyzing the interrelationship of social stratification, job conditions, and psychological functioning. (2) Using the data from both the men and their wives, analyzing the relationships between housework and psychological

functioning. (3) Continuing the analysis of the cross-national comparative study of Polish and U.S. data, with the focus now on life-course analyses of the relationship of education and of occupational self-direction to intellectual flexibility. (4) Analyzing a thoroughgoing replication, conducted in Japan, of the U.S. findings on the relationship of job conditions and psychological functioning. (5) Using the data for children, assessing the processes by which the educational process affects psychological development.

1. The relationship of social stratification and job conditions to psychological functioning. This entire program of research was originally designed to answer two questions: What is the relationship of social stratification to psychological functioning? To what extent does this relationship result from the greater opportunity to exercise occupational self-direction enjoyed by men of higher social position? The original analyses, published in 1969, attempted to answer these questions with cross-sectional data, utilizing the most advanced methods of statistical analysis then available, exploratory factor analysis and analysis of variance. Melvin Kohn and Carrie Schoenbach have now readdressed these questions, using longitudinal data and newly developed methods of analysis that are much more appropriate to the issues -- confirmatory factor analysis and linear structural-equation causal analysis. The methods used in the original analysis did not take measurement error into account; of even greater importance, those methods assumed unidirectional effects of social stratification on occupational conditions and of occupational conditions on psychological functioning. The new methods, as applied to longitudinal data, permit much more accurate measurement of key concepts. Moreover, they permit the analysis of reciprocal effects. In the new analysis, Kohn and Schoenbach assess as reciprocal the relationships between social-stratification position and occupational self-direction and between occupational self-direction and psychological functioning, testing empirically issues that previously were treated only by a priori argument.

A second purpose of this analysis is to address questions about social class similar to those they ask about social stratification. By social stratification, they mean the hierarchical ordering of society; by social classes, they mean groups defined in terms of their relationship to ownership and control of the means of production. Since social class represents a theoretically powerful alternative conceptualization of the socio-economic organization of industrial society, they ask the same questions about social class that they ask about social stratification: What is the relationship of social class to values, orientations, and cognitive functioning? To what extent does this relationship result from the greater opportunity to exercise occupational self-direction enjoyed by those who are more advantageously situated in the class structure?

The investigators find consistent relationships between social-stratification position and values and orientations: The higher men's social-stratification positions, the more likely they are to value self-direction, for themselves and for their children, and the more likely they are to hold self-directed orientations to self and society. They now add to those original conclusions that the magnitudes of the correlations are considerably larger than the earlier methods of analysis had indicated and that the basic pattern of correlations holds not only for values and the particular facets of orientation originally examined, but also for more fundamental dimensions of personality -- self-directedness of orientation, distress, alienation, and ideational flexibility.

They confirm, too, that the psychological impact of social-stratification position is substantially attributable to occupational self-direction. This conclusion is based on an empirical assessment of the reciprocal relationship between occupational position and occupational self-direction. They find each to affect the other strongly, with education affecting both. As a result, the correlation of occupational position with occupational self-direction is near unity. Thus, a basic tenet of the original interpretation, that there is a close relationship between social-stratification position and occupational self-direction, is confirmed. Despite this high correlation, though, it is possible (by disaggregating social-stratification and occupational self-direction into their component concepts) to distinguish the psychological effects of social-stratification position from those of occupational self-direction. These analyses demonstrate that the psychological impact of social-stratification position (and of its components, education and occupational position) is attributable, in very substantial degree, to occupational self-direction.

The original analysis posited that the experience of occupational self-direction must actually affect values and orientation. That proposition was both the crucial element in the entire interpretation and -- since it was based only on a priori argument -- the most questionable. Now it is possible to assess the reciprocal relationships between occupational self-direction and the several facets of values, orientation, and cognitive functioning. In every instance, occupational self-direction does have a causal impact on psychological functioning. In most instances, the relationship is reciprocal, with values, orientations, and cognitive functioning also affecting the exercise of self-direction in work. The interpretive chain is now complete: Social-stratification position affects and is affected by occupational self-direction; occupational self-direction affects and is affected by psychological functioning. Moreover, occupational self-direction, ideational flexibility, and a self-directed orientation are intertwined in a dynamic process through which the individual's place in the stratification system both affects and is affected by his personality.

In these analyses, Kohn and Schoenbach have also re-evaluated the processes by which education affects values and orientation. The models necessarily treat the effects of education on psychological functioning as unidirectional, hence undoubtedly exaggerate those effects. Still, the models tell us a great deal about process. Contrary to the hypothesis originally advanced, ideational flexibility does not play a substantial intervening role in the process by which education affects values and orientation. Instead, ideational flexibility is more affected by, than a determinant of, self-directed values and orientations. This being the case, another hypothesis -- that ideational flexibility plays an intervening role in the process by which occupational self-direction affects values and orientations -- also falls by the wayside. They conclude instead that the effects of both education and occupational self-direction on self-directed values and orientations are predominantly direct; and, furthermore, that the effects of education and of occupational self-direction on ideational flexibility are in part indirect, through self-directed values and orientations.

Finally, they learn that occupational self-direction plays a crucial role in explaining the psychological impact of social class, just as it does in explaining the psychological impact of social stratification. The psychological effects of social class examined in the analyses prove to be mainly a function of the varying degrees of occupational self-direction enjoyed by men at various locations in the class system. Perhaps occupational self-direction does not play so pivotal a role in explaining the impact of social-class position on such things as social-class identification and political ideology. But, for the psychological phenomena they have examined, occupational self-direction clearly plays a major part in explaining the impact of social class.

2. Housework and psychological functioning. On the hypothesis that the psychological impact of the conditions of work encountered in household work would be similar to those of job conditions encountered in paid employment outside the home, Kohn and Schooler included in the 1974 follow-up survey a set of questions about the nature of the actual work performed by the respondent in taking care of the household. Insofar as possible, these questions exactly parallel those asked about work performed in paid employment. The household work questions were asked not only of housewives, but also of working women and of men.

With this information, Carmi Schooler, Melvin Kohn, Joanne Miller, and Karen Miller have in past years developed measurement models of the basic conditions of work encountered in housework and have done multiple-regression analyses of the relationships between housework and psychological functioning. These analyses provided evidence that women's psychological functioning is related to the housework they do. Ideational flexibility is positively related to doing substantively complex housework and negatively related to doing housework that is heavy, dirty, repetitive

or in which the woman believes she is likely to be held responsible for things outside her control. Self-directedness of orientation is positively related to doing substantively complex housework and negatively related to doing dirty housework. Distress is related to doing housework under felt pressure of time or under circumstances where a woman believes she may be held responsible for things outside her control. All these findings are consonant with the possibility that women's housework affects their psychological functioning.

Although it is plausible that the multiple-regression findings result in substantial part from housework actually affecting psychological functioning, the direction of causal effects is far from certain. It could be argued that the relationship between housework and psychological functioning results mainly from women's personalities shaping the way they do their housework. To appraise causal direction, it is necessary to use linear structural-equation modeling to estimate possible reciprocal effects.

This year the causal analyses were completed. The results support the belief that the relationships found between household work and psychological functioning result in large part from housework affecting psychological functioning. Even though limited by the absence of longitudinal data, these reciprocal-effects analyses support the tentative conclusions of the earlier multiple-regression analyses. In particular, doing substantively complex housework results in increased ideational flexibility and a more self-directed orientation, while doing heavy housework results in a diminution of both.

Also corroborating the earlier multiple-regression analyses, the causal analyses of men's housework and psychological functioning indicate that it is not the substantive complexity but the heaviness of housework that is of central importance. The psychological effects of heavy housework on men seem more similar to those of substantive complexity than to those of heavy work in paid employment. The explanation of these effects of heavy housework is not readily apparent. One possibility is that physical exertion in an off-the-job context facilitates both intellectuality and a sense of efficacy, while sedentariness is detrimental to both.

Even more puzzling than the findings about the psychological effects of the heaviness of men's housework, is the absence of any significant findings for substantive complexity. In contrast to earlier analyses involving the substantive complexity of women's housework, or of men's or women's paid employment, the substantive complexity of men's housework ceases to be significantly related to their psychological functioning when other housework conditions are statistically controlled. The most likely explanation of this finding lies in the differential meaning of housework for men and for women. The evidence suggests that for many women household

work has much the same demand characteristics as does the work required in paid employment; thus, housework has structural imperatives whose psychological effects are similar to those of the structural imperatives of paid employment. For most men, in contrast, housework exerts no such imperative and thus does not have psychological effects similar to those of paid employment. If this interpretation is correct, the absence of significant relationships between the substantive complexity of men's housework and their psychological functioning, even though unanticipated, can be seen as consistent with the general interpretation. It may only be when it is imperative that work demands be met that the conditions of work have psychological effects.

In a more extended analysis of the nature of housework, Schooler, Karen Miller, and Joanne Miller also carried out a series of descriptive analyses of the work performed for the household and for pay, comparing husbands to wives and employed wives to full-time homemakers. In addition, husbands' and wives' perceptions of their household responsibilities were examined. These descriptive analyses show that the working conditions of housework do not differ greatly from the working conditions of the average paid job. As expected, however, there are marked sex differences in spheres of responsibility for and performance of specific tasks. Men's housework tends to be limited to household repairs, heavy work, and balancing the checkbook, while women are believed to be responsible for and actually do a wider range of household tasks.

3. The Polish replication. The main purpose of this inquiry is to examine the interrelationship of social stratification, job conditions and psychological functioning in a socialist society. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Previous Annual Reports reviewed their development of methods designed to assure cross-national comparability of indices and their analysis of two of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? If so, do these relationships result from the greater opportunities for occupational self-direction enjoyed by men of higher social-stratification position? As reviewed in detail in last year's Annual Report, the answers to both questions are positive with respect to values and social orientations. But higher social stratification position does not make for greater self-confidence in Poland (as it does in the United States). Nor does occupational self-direction affect self-confidence in Poland.

In further research this year, the analysis has focused on the determinants of intellectual flexibility at different stages of career. In this research, Joanne Miller, Kazimierz Slomezynski, and Melvin Kohn investigate intellectual flexibility through the life course using the national survey data from both Poland and the United States. The study focuses on how job conditions and education relate to effective intellectual functioning for men in early, middle, and late stages of their careers.

There are several reasons to expect variability in these relationships. Although previous research demonstrates that education and work conditions that require initiative and independent judgment promote intellectual flexibility, abilities may be harder to maintain or may change more slowly with age. It is also possible that successive birth cohorts may respond differently to these conditions either because of different capacities for intellectual growth or because of differences in the nature of their work and educational experiences. These alternative explanations cannot be resolved with either the American or Polish data alone. But, by making cross-national comparisons, some assessment of the universality and generalizability of these relationships can be made.

The analysis is divided into two major phases: 1) assessing the comparability of measured concepts across the life course as well as cross-nationally, and 2) testing of causal models. The first phase of the analysis has just been completed.

Intellectual flexibility is measured by five indicators reflecting intellectual performance in the interview situation: two tests of problem-solving ability, the Embedded Figures Test requiring ability to analytically and perceptually differentiate figure from ground, tendency to systematically agree with agree-disagree questions, and the interviewer's rating of overall intelligence. The investigators developed structural-equation measurement models to test whether these indicators be universally used to measure intellectual flexibility. Their analyses demonstrate substantial comparability regardless of age or nationality.

However, two interesting, if small, differences are found. First, the tendency to agree with agree-disagree questions is a stronger indicator of ideational flexibility in the United States than in Poland. This finding is consistent with the a priori belief that in Poland the interview situation is culturally defined as a formal interaction. Polish respondents may try to avoid disagreement in such a context. Thus, in addition to reflecting intellectual functioning, this intellectual functioning, this indicator also appears to reflect a particular cultural orientation towards the interview situation. Second, Polish interviewers appear to evaluate the overall intelligence of respondents on somewhat different criteria depending on the age of the respondent. For older men, performance on the tests of ability in the interview strongly influence the

interviewer's assessment of intelligence. For younger men, factors other than demonstrated performance influence the interviewer's rating. It is not possible to determine if these exogenous criteria are legitimate indicators of intelligence or whether they reflect irrelevant characteristics such as physical attributes. In any case, no such pattern is found in the American data.

In all, while some differences in measurement exist, they are of minimal importance. The correlations among scores based on several alternative models of ideational flexibility are high and the statistical fits of these models to the data are all acceptable.

Having established confidence in the measures indexing intellectual flexibility, the investigators next assessed the average competencies of workers at different stages of the life course. Three age groups were examined: men 30 years of age or younger, men 31 to 45 years of age, and men aged 45 or older. Both the American and Polish study confirm that, on average, older men are less intellectually flexible than are younger men.

In the second phase of this study, Miller, Slomczynski, and Kohn will investigate how job conditions and education affect the intellectual flexibility of workers at different stages of career. The analysis will focus on three job conditions reflecting occupational self-direction: substantive complexity, closeness of supervision, and routinization. As with the index of intellectual flexibility, they have developed age-specific measurement models of these concepts in each country. Here, too, while there is some variation in the structure of the models across age groups and between countries, the correlations among differing models are all high and they all fit the data reasonably well.

The investigators expected that levels of occupational self-direction would be, on average, higher for men at later stages of their careers than for men at earlier stages of their careers. Both in Poland and the United States, young workers are found to be less self-directed in their jobs than are middle-aged and older workers. However, the advantage of age subsides after the middle years; either there is no difference in average levels of self-direction between men at middle and late stages of career or middle-aged men have the advantage.

Thus far, the investigators have established that both intellectual flexibility and job conditions vary depending on stage of life course. In the next phase of this project they assess their interrelationships.

(4) The Japanese replication. Another major replication of the Kohn-Schooler survey has been conducted in Japan by Atsushi Naoi and Ken'ichi Tominaga of the Department of Sociology of Tokyo University. Data collection took place during the summer and fall of 1979. At that time, a probability sample of more than 800 employed men was interviewed with a

questionnaire containing all the questions necessary for indexing job conditions and those aspects of psychological functioning measured in the original study. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work collaboratively with Carmi Schooler. Initially, confirmatory factor analysis was used to develop measurement models for occupational self-direction, intellectual flexibility, parental values and several facets of self-conception and social orientation. These measurement models proved to be generally similar to those that had previously been developed for the American sample.

This year saw the completion of one of the major goals of the study -- the analysis of the causal relationship between occupational self-direction and psychological functioning. The central result is the generalization to Japan of the American findings. Occupational self-direction is found to have significantly positive effects on ideational flexibility, personally responsible standards of morality, and trust, and to have significantly negative effects on authoritarian conservatism, idea conformity, fatalism, and self-deprecation. In Japan, as in the United States, occupational self-direction leads to ideational flexibility and a self-directed orientation to self and society. As in the U.S., ideational flexibility and a self-directed orientation also reciprocally affect occupational self-direction.

The analyses also show a more extended relationship in Japan than in the U.S. between position in the organizational structure and psychological functioning. The causal models strongly suggest that in Japan ownership, hierarchical level and bureaucratization positively affect self-esteem. All three aspects of organizational position lead also to greater authoritarian conservatism. The comparatively greater authoritarian conservatism of Japanese in favorable organizational positions may be both a means of asserting their authority and a reflection of the attitudes of others. Comparison of the Japanese and American measurement models indicates that authoritarian conservatism in Japan is marked with a higher degree of obeisance and respect for those in authority than is authoritarian conservatism in the United States. In Japan, hierarchical level also results in more idea-conformity and standards of morality emphasizing pragmatic obedience rather than acceptance of personal responsibility for one's actions. This tendency of high position to lead to a conformist orientation indicates that "favored" occupational conditions do not necessarily lead to a self-directed orientation.

During the year, preliminary analyses of the relationships of social stratification to self-conception and social orientation in Japan were also carried out. These analyses confirm that, with one exception, the relationships of social stratification with social orientations and self-conceptions are similar in sign, albeit sometimes smaller in magnitude, than those found in cross-sectional analyses of data from the United States. The exception is that, in Japan, there is little if any relation-

ship between social-stratification position and anxiety; it may even be that men of higher social-stratification position are more anxious than are men of lower position.

Analyses were also begun on the role of occupational self-direction in explaining the relationship of social stratification to psychological functioning in Japan. Because the analyses are still in progress, the conclusions remain provisional. At this stage, occupational self-direction appears to explain, in substantial part, the relationships of social stratification to ideational flexibility, to social orientations and to those aspects of self-conception that are related to social stratification.

Taken together, these analyses substantially reinforce the impression that, although meaningful cross-national differences exist, the Kohn-Schooler interpretation of the interrelationships of social stratification, job conditions and psychological functioning is generalizable to Japan. Although the Japanese analyses have not been completed, there is now evidence that in Japan and the U.S. psychological constructs have essentially the same measurement characteristics and are (with the exception of anxiety) similarly related to social stratification. In both countries, the magnitudes of these relationships are substantially reduced when occupational self-direction is statistically controlled. In each country, occupational self-direction can be shown to affect those aspects of psychological functioning that are related to social stratification. Thus in Japan, as in the United States, the individual's position in the social stratification system affects his psychological functioning in large part because it embodies systematically different conditions of work that profoundly affect his personality.

5. Educational experience and children's psychological development.

The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis is to examine the processes by which students' educational experiences, particularly the degree of self-direction they are able to exercise in their educational endeavors, affect their psychological functioning. Data for this analysis were collected in the 1974 follow-up survey as part of the interview with children of the primary respondents. The interview schedule for these "children" -- now aged 13 to 25 -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions are designed to parallel those previously found to be powerful for analyzing occupational experience, focusing on such dimensions of the educational experience as its substantive complexity, how closely it is supervised, time-pressure, and the like. The intent is to see whether the concepts and methods developed for the study of occupational experience can be applied as well, and with similar results, to the study of educational experience.

Part of this year's work was devoted to refining the measurement model of "educational self-direction," a preliminary version of which was developed in 1980-81. "Educational self-direction" is directly analogous to occupational self-direction; it is defined to mean the use of

initiative, thought, and independent judgment in schoolwork. In the refined model, the substantive complexity of schoolwork and the absence of close supervision by teachers are the major indicators of this concept.

Another important part of this year's work was the development of a measurement model of intellectual flexibility for students, a model exactly comparable to that for adult men and women. The index is not intended to reflect innate intellectual ability or scholastic achievement; it does reflect students' actual intellectual functioning in a situation (i.e., the interview) outside the school context. Intellectual flexibility is conceived as incorporating two factors -- perceptual and ideational flexibility -- with the latter being the major focus of the research.

The main thrust of the analysis has been to develop and test a causal model of the reciprocal effects of educational self-direction and the ideational component of intellectual flexibility. The investigators encountered the usual difficulties associated with attempting such an analysis without longitudinal data. One tremendous advantage for this particular analysis, however, is the availability of information about the students' parents' psychological functioning. This means that, in examining the relationship between educational self-direction and any facet of psychological functioning, it is possible to statistically control both parents' levels of functioning. Thus far, one provisional model has been developed -- a model of the reciprocal effects of educational self-direction and ideational flexibility, taking into account parental ideational flexibility, age, sex, and school grade of the child, the extent to which the child's school courses are compulsory or elective, and pertinent social characteristics of the child and the family.

Preliminary results suggest that educational self-direction, in particular the substantive complexity of schoolwork, has a causal impact on students' ideational flexibility. It thus appears that, even in competition with the powerful genetic and environmental effect of parents' intellectual functioning and the powerful developmental effect of age, substantively complex schoolwork increases a student's ideational flexibility. During the coming year, the investigators will refine this model and further test this provisional conclusion. In addition, they will develop measurement models of other aspects of psychological functioning, such as self-directedness of orientation, distress, and values, testing their reciprocal relationships with educational self-direction.

Significance of the research:

This research is significant to the mission of the Institute on two distinct levels: (1) It has been well established that the incidence of schizophrenia is inversely related to social-stratification position. This relationship is not simply a function of greater genetic vulnerability at

lower stratification levels or of more stressful life conditions at those levels. In larger part, it seems to result from people at lower stratification levels having less effective psychological mechanisms for coping with stress and uncertainty. This research, at a very basic level, is investigating what there is about the conditions of life associated with social-stratification position that results in people of lower social-stratification position having less effective mechanisms for coping with stress and uncertainty. (2) Above and beyond its interest in mental disorder, per se, the Institute has a mandate to study the conditions that facilitate and those that interfere with effective psychological functioning. This research has demonstrated that job conditions have appreciable effects on cognitive functioning, self-conceptions, and orientations to the outside world. Much of the recent work in this research project has focused on (a) demonstrating that job conditions actually do have a causal impact on effectiveness of psychological functioning and (b) elucidating the processes by which job affects psychological functioning.

Proposed course of further research:

As is evident above, the analysis of the Polish and Japanese replications is incomplete, with much more to be done. The analysis of education, too, is far from complete. In addition to completing these analyses, the investigators intend to embark on a new phase of the overall research program, an analysis of the processes by which parents' values and practices affect the values and personality development of their children. There are data in both the U.S. and Polish studies with which to carry out such an analysis. Kohn has developed a provisional model of these processes and preliminary analyses have begun.

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Miller, J., Slomczynski, K.M., and Schoenberg, R.J: Assessing Comparability of Measurement in Cross-national Research: Authoritarian-Conservatism in Different Sociocultural Settings. Soc. Psychol. Q. 44: 178-191, 1981.

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- Kohn, M. L., and Schooler, C.: Job Conditions and Personality: A Longitudinal Assessment of their Reciprocal Effects. Amer. J. Soc. 87: 1255-1280, 1982.
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- Schooler, C.: Psychological and Social Perspectives on Status Attainment. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)
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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 00679-02 LSES |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Structural Equation Models in the Analysis of Data with Measurement Error | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Ronald J. Schoenberg Research Sociologist LSES NIMH | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Socio-environmental Studies | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1 | PROFESSIONAL: 1 | OTHER: |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this work is to further develop the methods and techniques for the <u>specification</u> and <u>estimation</u> of the parameters of <u>structural equation models</u> of survey data that contain random and nonrandom <u>measurement error</u> . Included in this are methods for the <u>identification</u> of the models, estimation of the means of unobserved variables, the determination of <u>model condition</u> , and the treatment of <u>polytomous variables</u> . | | |

Project Description:

The key analytical instrument of research in the laboratory is a computer program written and maintained by Ronald Schoenberg called MILS (Multiple Indicator Linear Structural analysis). The capabilities of this instrument has been expanded during the year. A new technique was developed by Schoenberg for the analysis of latent product variables (latent variables that are products of other latent variables) which allows for the estimation of parameters of quadratic equations in which the variables contain measurement error. The estimation of the parameters of models with product latent variables was impossible before. This technique has been incorporated into the MILS by Schoenberg. Two Generalized Least Squares (GLS) methods have also been added to MILS, accompanying the Maximum Likelihood (ML) method previously available. GLS requires fewer assumptions and therefore allows the estimation of models in which the available data fail to conform to the multi-normality assumption required for the ML method. Other investigators have become interested in MILS and the improvements added to it. About twenty universities have requested and have received this program, and are using it in their research.

During the year Schoenberg began the extension of latent variable techniques to the analysis of observed polytomous variables (polytomous variables are variables that are measured in a usually finite number of discrete categories). Methods were developed to analyze binomially distributed and poisson distributed data. Observed variables of this type present major difficulties when applying the usual methods. There have been recent advances in computer technology and statistics, however, which provide the basis for the solution of these problems. These new procedures have been applied to problems under investigation in this and other Laboratories and they show an improvement over traditionally used techniques.

In addition to helping the laboratory keep up-to-date on research methods--through personal consultation as well as by means of a weekly one and half hour class on research methods--Schoenberg has assisted investigators in other Laboratories and in other parts of the Government in updating and improving their research methods. He has assisted in the development of methods for the analysis of the acute effects of alcohol on intellectual functioning in a study supported by NIAAA and NIMH, of the effects of parent's behavior on infant's behavior for an investigator in NICHD, of a measurement model of commuter's attitudes for an investigator in DOT, of a model of alcohol use, aging, and mental ability for an investigator in the National Institute of Aging, and of a model of health care for an investigator in the Health Care Financing Administration.

Significance of the research:

The statistical methods and the computer program (MILS) that have been developed in the course of this project are fundamental to the research program of the Laboratory of Socio-environmental Studies, for they enable the investigators to deal straightforwardly with the two most important methodological problems faced in studying the effects of social structure on personality: how to deal with measurement error and how to assess the direction of causal effects. These techniques are also proving to have considerable value to other Laboratories within the Intramural Research Program of NIMH and other Institutes of NIH and ADAMHA. Current work is designed to solve further statistical and methodological problems faced by intramural investigators and to make the computer program even more valuable.

Proposed course of further research:

During the coming year Schoenberg will be continuing his work on the analysis of polytomous variables. The weekly seminar will, beginning September, focus on the log-linear analysis of dichotomous variables and will later extend to the new, more powerful methods being discussed in the statistics journals--the Rasch models where a subject responds to several dichotomous items with different difficulties; multivariate probit models, i.e., threshold models; and the hazard rate models of Cox which are hybrids of log-linear models and stochastic processes. In addition he will be extending these basic ideas and developing them for application to the particular problems faced by the investigators in this and other Laboratories

Publications:

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Schoenberg, R.J., Parker, E.S., Parker, D.A., and Brody, J.: Cognitive Patterns Resembling Premature Aging in Male Social Drinkers in Alcoholism: Clin. Exp. Res. 6: 46-52, 1982.

Project Description:

Objectives: This long-term investigation is concerned with identifying brain mechanisms underlying species-typical forms of communication. For communication, terrestrial vertebrates engage in four main kinds of displays that may be characterized as (1) signature, (2) challenge, (3) courtship, and (4) submissive displays. In addition to dynamic modifiers, such displays may include static modifiers largely dependent upon autonomic function. Squirrel monkeys were chosen for the experiments involving the use of primates. The highly predictable mirror display of the gothic-type squirrel monkey is especially suitable for systematically testing the effects of cerebral lesions on the somatic and autonomic components of a species-typical display. The mirror display of these monkeys combines features of their challenge, courtship, and signature displays. The major outcome of this investigation has been the finding that the medial pallidal segment of the striatal complex is a site of convergence of neural systems involved in the performance of the display. Electrocoagulation of this segment or one of its major projecting pathways results in elimination or fragmentation of the display. Since experiments involving the pallidotegmental projections might have damaged monoaminergic pathways ascending from the isthmus region, the present experiments are being undertaken to learn whether or not elimination of respective isthmical cell groups containing serotonin and norepinephrine affect the performance of the display.

Methods Employed: Squirrel monkeys are of two main varieties--those with an ocular patch forming a peak over the eye (gothic-type) and those with a rounded patch (roman-type). In addition to differences in appearance and behavior, the two varieties are distinguished by their karyotypes. Only the gothic-type monkey will consistently display to its reflection in a mirror. An individual mirror test is scored on the basis of five components--namely, vocalization, thigh spreading, penile erection, urination, and scratching. Monkeys are tested twice a day. After achieving criterion performance (full displays in 80 percent of 30 or more trials), a subject undergoes surgical ablation or electrocoagulation of a specific structure. After allowing several months for brain changes to stabilize and after an animal achieves plateau performance, experiments are terminated and brains are prepared for histological analysis and volumetric measurements of the amount of destroyed tissue.

Major Findings: In one monkey (Q-5), an electrode was stereotaxically placed for coagulating the superior nucleus of Bechterev, a ventral raphe nucleus having many serotonin-containing cells. Although this animal showed increased motor activity, the mirror display was unaffected over a period of six months. It is now being used for obtaining supplemental information regarding another structure (see below). In a second animal (R-4) an electrode was directed for coagulating nuclei in the dorsal isthmus. Because of variation in stereotaxic co-ordinates owing to the mesencephalic flexure, there is the possibility of coagulating either the dorsal raphe nucleus with serotonin-containing cells, the deep tegmental nucleus of Gudden, or the locus caeruleus with norepinephrine-containing cells. This animal showed no changes during two and one-half months of testing. It is now being used for the purpose described below.

Supplemental experiments.

1. Thalamic fasciculus. In earlier experiments involving the subthalamic tegmentum, there were no electrocoagulations restricted to that part of the thalamic fasciculus where it reverses course and ascends to the thalamus. One monkey with an electrocoagulation aimed at this locus continues after four months of testing to show a decline to the zero level of display vocalization, but no change in other components of the display.

2. Olfactostriatum. In earlier experiments, partial bilateral coagulations of the olfactostriatum had no effect on display behavior. In monkey Q-5, described above, an attempt was made to produce a more complete coagulation of the olfactostriatum. During the two months since this operation, this monkey has continued to perform the complete display.

3. Destruction of rostral limbic cortex. Monkey R-5 mentioned above is being used as a pilot animal in the study of the isolation call (see accompanying report #871-06) to test the effect of extensive removal of the rostral limbic cortex and the adjoining neocortex. In the four months since surgery there has been a fragmentation of the trump display manifest by a decline of vocalization to the zero level and a statistically significant decline in the thigh-spread component.

Significance to Biomedical Research and the Program of the Institute:

It has been a major purpose of this long-term investigation to identify brain mechanisms involved in the displays of animal communication. Previous work on this project has shown that the striatal complex (a phylogenetically ancient formation of the forebrain) plays an essential role in integrating the performance of displays. Mechanisms underlying autonomic components of displays are least understood, but can be linked, in part, to limbic structures of the forebrain. The isthmus region at the junction of the midbrain and pons contains neural mechanisms essential for the integration of somatic and visceral functions (e.g., respiratory, ingestive, reproductive) and in primitive vertebrates is largely under the control of the midbrain. With the evolutionary expansion of the forebrain in reptiles, the control of isthmic somatovisceral "centers" requires the laying down of new and long connections with the telencephalon. The existence of ascending isthmic monoaminergic systems was unsuspected until recent neuroanatomical techniques made it possible to demonstrate them. The present project is being undertaken not only to assess the role of serotonin- and norepinephrine-containing cell groups in the isthmus, but also the dorsal tegmental nucleus of Gudden, which may be involved in reproductive behavior, and which, in addition to containing substance P and enkephalin, may be a source of ascending cholinergic fibers.

Proposed Course: To be continued.

Publications:

MacLean, P.D.: On the Origin and Progressive Evolution of the Triune Brain. In Armstrong, E. (Ed.): Primate Brain Evolution: Methods and Concepts. New York, Plenum Publishing Corp., 1982, pp. 291-316.

MacLean, P.D.: Evolution of the psychencephalon. Zygon (in press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00787-03 LBEB |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Species-typical Behavior in Squirrel Monkey: The Isolation Call | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | Paul D. MacLean John D. Newman Michael R. Murphy Carroll R. Harbaugh Robert E. Gelhard Thalia K. Bussard | Chief Research Physiologist Guest Worker Biol. Lab. Tech. Biologist Biologist LBEB NIMH LDN NICHD LBEB NIMH LBEB NIMH LBEB NIMH LBEB NIMH |
| COOPERATING UNITS (if any) J.D. Newman, LDN, NICHD | | |
| LAB/BRANCH Laboratory of Brain Evolution and Behavior | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837 | | |
| TOTAL MANYEARS: 1.25 | PROFESSIONAL: 0.3 | OTHER: 0.95 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> In the evolution from reptiles to <u>mammals</u>, the development of <u>audiovocal communication</u> became of utmost importance in maintaining <u>maternal-offspring contact</u>, as well as contact of members of social groups. The <u>isolation call</u> (separation call) is perhaps the most primitive and basic of <u>mammalian</u> vocalizations. In preceding work on squirrel monkeys (Z01 MH 00787-01 LBEB) it was shown that <u>tegmental lesions</u> involving the gray matter at the junction of the <u>third ventricle</u> and <u>aqueduct</u> affects the patterning and production of the isolation call. Since the <u>rostral limbic cortex</u> is the main cortical region involved in the vocalization of monkeys and since it projects to the core gray matter in question, the present project is designed to assess the effects of ablating this cortical region on the production and regulation of the isolation call. In addition to the initial results of these experiments, the present report describes the findings in a subsidiary study concerned with the effects of <u>opiate drugs</u> on the production of isolation calls. </p> | | |

Project Description:

Objectives: In the evolution from reptiles to mammals the development of audiovocal communication became of vital importance for maintaining maternal-offspring contact and contact of members of a group. There is evidence that the transitional mammal-like reptiles (therapsids) living in Permian and Triassic times were hard of hearing and may have been mute like most existing lizards. The isolation call (separation call) might be ranked as the most basic mammalian vocalization, since it serves originally to maintain maternal-offspring contact. It has been recorded in all mammals thus far examined.

The overall purpose of the present project is to identify brain mechanisms involved in the production and patterning of the isolation call (see below regarding significance). In addition to the karyotypic and behavioral differences described in an accompanying report (#851-18), gothic- and roman-type squirrel monkeys can be distinguished by their isolation calls. Since the isolation call becomes stable by one year of age, and since it can be readily elicited under experimental conditions, it lends itself to testing the effects of brain lesions on the production of the call. In preceding work on this project, it was shown that tegmental lesions involving the gray matter at the junction of the third ventricle and aqueduct affects the patterning and production of the call. Other vocalizations were unaffected. Since the rostral limbic cortex is the main cortical region involved in the vocalization of monkeys, and since it also projects to the core gray matter in question, it is the purpose of the present experiments to test the effects of rostral limbic ablations on the production and regulation of the isolation call. As will be described, two subsidiary aspects of the present project deal with (1) a comparative study of vocalizations produced during displays and (2) testing the effects of opiate-related drugs on the isolation call.

Methods Employed: Gothic- or roman-type of more than one year of age and of either sex are subjects for the present study. Isolation calls are induced by placing a monkey alone in a sound-attenuating chamber. Recordings are obtained with a standard Uher microphone and recorder over a period of 30 minutes. A VII model 700 sound spectrograph is used for spectrographic analysis.

Major Findings: The strategy in the initial experiments is to aspirate the total limbic cortex in question, together with the neocortical areas that are subject to injury during the surgical approach. The limbic areas include the cortex of the rostral part of the cingulate gyrus, the subcallosal gyrus, and the posterior part of the gyrus rectus. Given a positive result, an attempt will be made to identify the critical cortical area or areas. One monkey (R-5 used also in Project #815-18), in which the total cortex in question appears to have been aspirated, has failed to produce spontaneous calls during 20 weeks since operation. Another monkey (#914) with a less extensive lesion, and in which the rostral supracingular cortex and that of the posterior gyrus rectus were left intact, produced no calls during the

first post-operative week, but thereafter regained her pre-operative level of performance.

Additional experiments. Two monkeys (Q-5 and S-5) used in the display studies (see accompanying report #851-18) are also subjects in the present experiments. Each has produced spontaneous isolation calls, but below criterion, during four months of testing.

Comparative observations on mirror display vocalizations. Since the mirror display vocalizations incorporate sounds resembling the isolation call, we are undertaking a comparative analysis of spectrograms recorded during displays. Thus far, an analysis involving five unoperated adult squirrel monkeys has shown that sounds emitted during displays are of three main types: (1) broad-band, (2) tonal, and (3) rapid frequency-modulated. Four monkeys (#928, 933, Q-5, R-5) emitted tonal calls resembling isolation calls, but in each case the rising phase was greater in amplitude and in three (#928, Q-5, and R-5) the tonal frequency was 2 kHz below that of their isolation calls. The calls were frequently fragmented or contained noisy (or fricative) components characteristic of isolation calls of immature monkeys. A fifth monkey (S-5) emitted only chucks during displays.

Effects of opiate-related drugs. The core gray matter involved in the isolation call (see Objectives) is continuous with the central gray surrounding the aqueduct, a region known to be basically involved in vocal and "pain" mechanisms and recently shown to have a high concentration of opiate receptors. Experiments were conducted on four female squirrel monkeys (#576, 934, 917, C-1). In following a protocol involving several controls, it was found that the administration of 10 mg/kg of morphine sulphate resulted in the cessation of isolation calls, whereas such calls recurred after parenteral injections of 0.5 mg/kg of naloxone. The findings are in agreement with those of Panksepp et al. in experiments on guinea pigs and dogs.

Significance to Biomedical Research and the Program of the Institute: In the light of the great dependence of human communication on both emotional phonation and propositional speech, it is of fundamental interest to obtain information about the evolutionary acquisition of brain mechanisms of vocalization in mammals. In the present work on squirrel monkeys we have chosen to focus on identifying mechanisms underlying the isolation call (separation call) because it would appear to be the most primitive and basic mammalian vocalization, serving to maintain maternal-offspring contact.

The separation calls of human infants have features resembling those of the great apes and monkeys. The results of the present project promise to be of value in the clinical diagnosis of vocal abnormalities, as well as in indicating the location of brain structures responsible for disordered function.

The separation calls of human infants are frequently referred to as distress calls. In view of the distress element, it is of interest that a subsidiary study of the present project has shown that the administration of

morphine to squirrel monkeys results in the cessation of isolation calls, whereas such calls recur after treatment with naloxone.

Proposed Course: To be continued.

Publications:

Newman, J.D.: The Infant Cry of Primates: An Evolutionary Perspective. In Lester, B.M., and Boukydis, C.F.Z. (Eds.): Infant Crying: Theoretical and Research Perspectives. New York, Plenum Publishing Corp. (in press).

Newman, J.D., and MacLean, P.D.: Effects of tegmental lesions on the isolation call of squirrel monkeys. Brain Res. 232: 317-329, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00793-01 LBEB | | | | | | | | |
| PERIOD COVERED September 1, 1981 through October 31, 1982 | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Brain Iron and Neuroendocrine Regulation | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Joanna M. Hill</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">LBEB NIMH</td> </tr> <tr> <td>Other:</td> <td>Carroll R. Harbaugh</td> <td>Biol. Lab. Tech.</td> <td>LBEB NIMH</td> </tr> </table> | | | PI: | Joanna M. Hill | Visiting Fellow | LBEB NIMH | Other: | Carroll R. Harbaugh | Biol. Lab. Tech. | LBEB NIMH |
| PI: | Joanna M. Hill | Visiting Fellow | LBEB NIMH | | | | | | | |
| Other: | Carroll R. Harbaugh | Biol. Lab. Tech. | LBEB NIMH | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | |
| LAB/BRANCH Laboratory of Brain Evolution and Behavior | | | | | | | | | | |
| SECTION | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837 | | | | | | | | | | |
| TOTAL MANYEARS: 0.50 | PROFESSIONAL: 0.25 | OTHER: 0.35 | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Earlier studies in this laboratory have demonstrated a relation between <u>brain iron</u> and <u>neuroendocrine regulation</u> . The present experiments were designed to learn whether or not intraventricular injection of an <u>iron chelator (deferroxamine mesylate)</u> in the region of the <u>ventromedial hypothalamus</u> and <u>median eminence</u> would affect the <u>estrous cycle</u> . Estrous cyclicity was determined by the vaginal smear technique. Eight of 10 rats injected with iron chelator exhibited a loss of estrous cyclicity of 2 to 4 cycles duration, whereas only 2 of 10 saline injected controls had a period of acyclicity ($p < 0.01$, Fisher's exact test). The <u>iron histochemical</u> and histological examination of brain tissue is now in progress. | | | | | | | | | | |

Project Description:

Objectives: This project is part of the concerted effort in this laboratory to clarify the functions of the striatal complex. On the basis of findings described in last year's progress report (Z01 MH 00846-03 LBEB) there were the following indications that brain iron plays a role in the control of neuro-endocrine regulation: (1) the amount of brain iron is greater in the female than the male (demonstrated both histochemically and quantitatively); (2) the amount of iron in the globus pallidus and substantia nigra almost doubles at proestrus; (3) a significant rise also occurs during the first third of pregnancy; and (4) iron is located histochemically in many brain sites known to be estrogen sensitive and/or having an influence on pituitary regulation. It is especially noteworthy that iron is present in the organum vasculosum of the lamina terminalis and in the tanycytes of the ventromedial hypothalamus and median eminence.

The present study was designed to learn whether or not the injection of an iron chelator, desferoxamine mesylate, into the third ventricle in the region of the ventromedial hypothalamus and median eminence would affect the estrous cycle.

Methods Employed: After demonstrating the regularity of the estrous cycle by the vaginal smear technique, 10 female rats received an intraventricular injection of 3.0 μ l of a 0.1% solution of the iron chelator, desferoxamine mesylate. A control group of 10 females were injected with an equal amount of saline in the same region. Vaginal smears were taken daily for at least two weeks following the injection. All animals were sacrificed at diestrus and perfused with formol-saline. The brains were removed and cut on a freezing microtome at 25 and 50 μ m and stained for iron with diamino-benzidine intensified Perl's reaction for ferric iron and counterstained with thionine.

Major Findings: Within two days after treatment, 8 of 10 animals in the experimental group and 2 of 10 controls became acyclic ($p < 0.01$, Fisher's Exact Test). The period of anestrus persisted for the equivalent of 2 to 4 cycles, after which all animals resumed normal cycling patterns. In the brains thus far examined the histochemical examination reveals a suggestive paling of the iron stain in the tanycytes of the median eminence of the desferoxamine mesylate-treated animals.

Significance to Biomedical Research and the Program of the Institute: The results of the present experiments suggest a functional link between brain iron in the hypothalamus and the estrous cycle of the female rat. In particular, the findings indicate that the temporary sequestration of iron by chelation may affect neuroendocrine regulation at the level of the hypothalamus where iron is present in tanycytes. The following considerations suggest that the tanycytes are implicated in neuroendocrine regulation: (1) tanycytes have been reported to undergo morphological changes throughout the estrous cycle; (2) they contain LHRH and serotonin;

(3) they receive terminals of dopamine-containing fibers; and (4) they furnish a functional link between the third ventricle and the hypophyseal portal system. The iron in the tanycytes may be related to some aspect of the metabolism of the peptide luteinizing hormone-releasing hormone (LHRH) or to that of the monoamines.

Iron deficiency is the most prevalent nutritional disorder of the human population. It is evident that the findings of the present study may prove relevant to the clinical conditions of amenorrhea and menstrual irregularity in cases of iron deficiency.

Proposed Course: To be continued.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00871-06 LBEB |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | |
| TITLE OF PROJECT (80 characters or less) A Histochemical Study on the Location of Brain Iron | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Joanna M. Hill Visiting Fellow LBEB NIMH | | | |
| COOPERATING UNITS (if any) | | | |
| LAB/BRANCH Laboratory of Brain Evolution and Behavior | | | |
| SECTION | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837 | | | |
| TOTAL MANYEARS: 1.20 | PROFESSIONAL: 0.85 | OTHER: 0.35 | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Light microscopic examination of brain tissue prepared with an improved <u>histochemical method</u> (diamino-benzidine intensified <u>Perl's reaction</u> for <u>ferric iron</u>) has revealed that many brain areas concentrate iron, but that the type of cell in which iron accumulates and the degree of accumulation differ and are characteristic for each area. In the present study the histochemical method has been further modified for identifying the distribution of <u>brain iron</u> at the electron microscopic level. <u>Electron microscopy</u> permits the identification of (1) the type of cell concentrating iron, (2) the location of iron with respect to the cell body and processes of <u>neurons</u> and <u>glia</u>, and (3) the <u>cellular organelle(s)</u> in which iron accumulates. The acquisition of such information is requisite for gaining an understanding of the function brain iron.</p> | | | |

Project Description:

Objectives: This project was initiated in conjunction with other ongoing investigations in the laboratory regarding the functions of striatal structures of the basal forebrain. The globus pallidus of the striatal complex concentrates iron to a greater degree than other forebrain structures. The function of pallidal iron is unknown. Light microscopic investigation of brain iron has revealed that iron is present in fibers and glial cells. The question as to its presence in small neurones in the globus pallidus and in other brain areas is unanswered. In the circumventricular organs, iron appears as amorphous accumulations and granules; it is localized within tanycytes of the ventromedial hypothalamus and median eminence. In some brain nuclei, iron occurs in "bouton-like" structures or as small grains that appear to be located either upon or within the neurones and neuronal processes. The immediate purpose of the present electron microscopic study is to answer the following questions: (1) What types of glia or neurones concentrate iron? (2) Is the grain-like deposit of iron on the outer or inner surface of cells? (3) Is iron present in various organelles? Although the study will focus on the globus pallidus, ventral pallidum, and islands of Calleja, it is intended to obtain comparative information about the distribution of iron in circumventricular sites and in those areas in which iron is apparently localized primarily within neurones. As a first step, it has been necessary to modify the Perl's+DAB method for iron for electron microscopic use. The identification of the subcellular localization of iron is a requisite for understanding the functional role of brain iron.

Methods Employed: The Perl's+DAB histochemical method for ferric iron described in last year's progress report (Z01 MH 00871-06 LBEB) has been modified for electron microscopic use. Brain tissue is perfused with a standard electron microscopic perfusion mixture of glutaraldehyde and paraformaldehyde instead of formol-saline. The tissue is cut in 70-100 μ m sections on a vibratome at room temperature in a medium of cocodylate buffer. These thick sections are subjected to the usual Perl's+DAB reactions, except that tris buffer is substituted for phosphate buffer in the DAB mixture and the distilled water rinse between the Perl's reaction and immersion of the tissue into DAB extended to 30 min and the tissue refrigerated during this time. Selected samples from the sections are treated with 1 percent osmium tetroxide overnight, after which the tissue is rinsed in acetate buffer and stained with 0.5 percent uranyl acetate for 30 min on ice. After staining, the tissue is dehydrated, embedded, cut, and examined with an Hitachi HS 8 electron microscope.

Major Findings: The Perl's-DAB method for ferric iron has been successfully modified for locating brain iron with the electron microscope. The first electron micrographs have just been obtained. In the ventral pallidum it has been found that iron is present surrounding the axoplasm between the axoplasm and myelin and also in a thin layer on the outer surface of the myelin membranes.

Significance to Biomedical Research and the Program of the Institute:

The present electron microscopic study on the location of brain iron may be expected to provide information not only about cell types that accumulate iron, but also anatomical details relevant to metabolism and neural transmission. In an accompanying report (#00793-01), the relationship between brain iron and neuroendocrine regulation is discussed. The association of iron with many neurotransmitter systems suggests that it may have other special functions. As discussed in last year's report (#00871-05), the presence of iron overlaps that of GABA and such neuropeptides as LHRH, substance P, and enkephalins. Iron is also involved in numerous metabolic functions (see, e.g., below). as well as for serotonin-binding protein and serotonin receptor mechanisms.

In view of the high concentration of iron in the globus pallidus and substantia nigra, the function of brain iron must also be considered in regard to several neurological diseases (e.g., Huntington's chorea and Parkinson's disease, as well as disorders of metal metabolism, including Wilson's disease, Hallervorden-Spatz disease, and manganese poisoning). It should also be emphasized that pallidal and nigral iron accumulate with age. Finally, it is of special interest that changes in monoamine metabolism have been found in cases of schizophrenia and in manic-depressive states, a situation that recalls that iron is necessary for the synthesis and degradation of monoamines, as well as for the storage and receptor mechanisms involving serotonin.

Proposed Course: To be continued.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00847-02 LBEB |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Role of the Neocortex in Coping with Complexity | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: James L. Hill Other: Garrett A. Bagley Charles L. Bishop Etienne T. Lamoreaux Paul D. MacLean Thalia K. Bussard | Visiting Associate Computer Programmer Biologist Psychology Technician Chief Biologist | URBS LBEB NIMH URBS LBEB NIMH URBS LBEB NIMH URBS LBEB NIMH LBEB NIMH LBEB NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Brain Evolution and Behavior | | |
| SECTION Unit for Research on Behavioral Systems | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837 | | |
| TOTAL MANYEARS: 3.0 | PROFESSIONAL: 1.7 | OTHER: 1.3 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Micrencephaly</u> is induced in rats by injecting their mothers with <u>methylazoxy-methanol acetate (MAM)</u> during pregnancy. The areas of the <u>brain</u> most effected are the <u>cerebral hemispheres</u> showing marked <u>reductions</u> in both <u>neocortex</u> and <u>limbic cortex</u> . Rats with this chemically induced micrencephaly exhibit <u>species typical behaviors</u> and reproduce successfully in restricted laboratory cage environments which may present no significant challenge to the survival of micrencephalic individuals. The purpose of this study is to examine the behavior of micrencephalic rats living in slightly crowded, free-ranging groups in large, complexly structured environments: survival in these environments requires the learning of complex behaviors, such as lever pressing for water. Our preliminary results show that, although the <u>micrencephalic rats</u> can survive in mildly stressful situations, they exhibit a <u>drastic reduction</u> in <u>reproductive success</u> . Only about 10 per cent of the pups in the first litters born to MAM treated rats survived, compared to over 80 per cent of the pups in the first litters born to control rats. Poor reproductive success of the micrencephalic parents is associated with <u>poor maternal behavior</u> , and <u>high</u> levels of disruptive <u>aggressive behavior</u> among males. | | |

Project Description:

Objectives: Past studies of rats with experimentally induced micrencephaly characterized by a great reduction of the neocortex and limbic cortex have revealed remarkably few behavioral deficits. Such rats, however, have been observed only in greatly restricted environments. The object of the present study is to determine if micrencephalic rats with a great reduction of cortex are able to develop appropriate behaviors for surviving over an extended period while living as groups in complex environments.

Methods: On day 15 of pregnancy, female rats are injected with a 20 mg/Kg body wt. dose of methylazoxymethanol acetate (MAM). This chemical is converted in vivo to diazomethane which kills dividing cells by alkylating the purine and pyrimidine bases in their nucleic acids. Thus, in the 2 to 24 hr effective period of the drug, rapidly dividing neuroblasts in the cortical matrix of the fetus are destroyed and, the full development of the neocortex and limbic cortex is prevented. There are two MAM-treated populations and two control populations being studied at each of two levels of environmental and social complexity: (1) Eight bisexual pairs of rats are housed in each of four rooms (9x9x12 ft) of high structural complexity containing twelve pairs of nest sites located on a total of six shelves, arranged on two adjacent walls at heights of 3, 6, and 9 ft above the floor. (2) Four bisexual pairs are housed in each of four rooms (9x9x12 ft) of lower structural complexity rooms, containing six pairs of nest sites on two single shelves on opposite walls, 9 ft above the floor. In addition adult growth and reproductive performance of the rats in the rooms are being compared in MAM-treated and control bisexual pairs housed in individual laboratory cages.

Movement patterns of all ninety-six rats in the eight rooms are monitored by a computer controlled data acquisition system. Behavioral observations are made through a viewing window in the ceiling of each room. The ultrasonic vocalizations of experimentals and controls (isolated infants and of free-ranging adults) are being recorded and compared. Data on reproduction, physical condition, and social status (as judged by location and number of wounds), as well as the weight and condition of pups, are recorded during weekly surveys of all animals. Each individual's nest site and quality are recorded daily.

Major Findings: MAM induced micrencephalic rats are capable of surviving in social groups in complex environments for extended periods. Although there are no obvious differences between treated and control groups, we have observed the following deficiencies in the behavior of the treated animals: reduced neophobia and fear of humans, increased and inappropriate aggression, and poor maternal care. Deficient maternal care was reflected by poorer nest construction and choice of nest locations. As compared with the controls, the experimentals in the highly complex rooms were only 3% as effective in rearing pups from initial litters: in the less complex rooms the experimentals were about 20% as effective as the controls. The daily activity pattern of all animals follows the reversed light cycle with the treated animals moving between compartments more frequently. Ultrasonic vocalizations are emitted by both treated and control animals both as isolated pups and as free-ranging adults. Although not scored, play behavior was observed in treated pups but it was not as frequent or as vigorous as in the controls.

Preliminary histological examination of the brains of siblings of treated animals indicates that the entire cerebral hemispheres are markedly reduced; the neocortex and limbic cingulate cortex show the greatest reduction in area and disruption in layering pattern. The hippocampus, however, is fairly well developed. The corpus collosum is absent except for the part above the septum. The thalamus is less reduced in size than one would expect in view of the massive loss of cortical tissue. The midbrain cerebellum, and medulla appear to be little affected.

Significance to Biomedical Research and the Program of the Institute:

This project involving experiments, in which the cortical development in rats is massively reduced, represents a model system for observing how micrencephalic mammals deal with complex environments and social situations. This research will also permit an assessment of the degree to which species typical behavior is regulated by cortical areas. Such experiments have obvious relevance to problems pertaining to mental retardation.

Future Course: The animals will be removed from the environments during the next few weeks, and the brains prepared for histological examination. The analysis and comparison of behavioral and histological data will be conducted in the following months, and the project concluded with the preparation of the results for publication.

Publications: None.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00848-01 LBEB |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Influence of Environmental Setting on Behavior and Population Dynamics. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: John B. Calhoun Other: James L. Hill Garrett A. Bagley Charles L. Bishop Etta Maye Zoerb | Research Psychologist Visiting Associate Computer Programmer Biologist Editorial Assistant | URBS LBEB NIMH URBS LBEB NIMH URBS LBEB NIMH URBS LBEB NIMH URBS LBEB NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Brain Evolution and Behavior | | |
| SECTION Unit for Research on Behavioral Systems | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837 | | |
| TOTAL MANYEARS: 6.0 | PROFESSIONAL: 3.5 | OTHER: 2.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) A coordinated set of studies has been completed through the initial phases of (1) preliminary analyses culminating in formulating a set of tentative insights to serve as a guide to preparing final analyses, (2) restructuring of computer file data bases to facilitate further analysis. Major conclusions from studying mice and rats (<u>Mus musculus</u> and <u>Rattus norvegicus</u>) in designed habitats: (1) <u>Behavioral deviance</u> begins to be significant at twice optimum density and by 8 times optimum density produces zero <u>reproductive success</u> , which is followed by <u>population extinction</u> , but isolation of reproductive-aged pairs permits sufficient behavioral recovery for successful rearing of progeny. (2) <u>Habitats</u> consisting of interconnected compartments, arranged to produce a bilaterally symmetrical <u>communication network</u> , enhance preservation of normality of individual behavior and <u>group structure</u> . (3) Provision of opportunity to develop <u>cooperative behavior</u> can largely offset deleterious consequences arising from increase in density. (4) Layered strategies employed by rats in consummating cooperative behavior suggest an increase in <u>conceptualization capacity</u> proportional to the increase in <u>population size</u> . | | |

Project Description

Objective: To determine how physical, particularly spatial, and social environmental variables influence the behavior of animals and the mental health of people in the context of group and population dynamics. Major focus is on three large scale studies pursued from 1974 through 1982 to the stage of preliminary analyses and structure of a 25-million item data base to provide easy access for computer facilitated detailed analyses. Primary emphasis will be on the two animal model studies of mice and rats in designed environments. Only sufficient remarks will be made about the human related study to show how thought behavioral states of humans evolve from behavioral states of lower mammals, and adhere to the same principles of CNS control.

Delineation of the objectives for these three studies, and the opportunity for their conduct, could only have arisen within the context of the NIMH. This support permitted pursuit of a basic precept: This is that experimental enquiry into problems related to group and population dynamics can only be fruitfully delineated from a broad understanding of the type of species to be studied derived from observations made over the natural settings in which they normally live. This conviction applies to humans as well as animals because the relevance of our research to the human scene can only be determined with reference to how humans function in their normal settings. The financial support for this dual objective has been equally borne by the intramural and extramural NIMH programs.

We have been able to conduct intensive studies of Norway rats and house mice, our prime subjects, as well as of similar ecological types of small mammals in their natural habitats. We have also developed communication networks over two subject areas with over 400 scientists whose work with animals dealt with life history studies and population censusing. Through these contacts we have been able to influence the initiation and design of a number of studies which produced conclusions that were crucial to our formulations of behavior and group dynamics.

Other communication networks, primarily providing knowledge about human settings, involved another 400 scientists, particularly in the behavioral sciences. The four major ones were: (1) The "Space Cadets" (Committee on Physical and Social Environmental Variables as Determinants of Mental Health): On the average 19 prominent persons representing a wide range of disciplines gathered twice a year for 2 1/2 days in each session for 13 years. The core group of 12 persons was supplemented by 7 guests each session, with each guest on the average participating in 7 sessions. 72 persons were involved. This effort was initiated by Calhoun. (2) The human environment: Reports prepared by us for, and at the request of, the HEW Secretary's Task Force on the Environment provided the philosophical structure of the Task Force's final report. The National Environmental Protection Act derived directly from this report. Participation in this effort opened up another communication network that proved of great value to defining objectives for our research program. (3) Information about the built environment relevant to the health and welfare of people: Based on a request originating from the Office of the President we structured a study on this topic and supervised its conduct under support from HUD, NIMH and NLM. This study coordinated the advice from 15 core consultants and 200 correspondents. (4) Important researchable problems related to adaptation, environment

and population: 162 prominent scientists provided us with detailed statements especially prepared for us. In addition, and derived from a request from the Director, NIMH, a survey was made of literature related to Population and Mental Health. This culminated in a cross-referenced anthology of 3,200 excerpts from 350 books and research papers.

These several avenues for gaining understanding about animals and humans living in their natural settings continuously fed back into our research program to modify research design and construct theory. Then out of the empirical research, and its contribution to elaboration of theory, a small core set of constructs emerged. Most important among these is the concept of optimum group size and its derivation from the consequences of requiring a fixed homesite which accompanies the long postnatal rearing period of progenitors of modern mammals. The relationship among our derived core constructs, and to the evolutionary/genetic demand for attaining optimum group size, is briefly reviewed below in order to show their importance to the objectives and design of our three crucial studies. We use the term "study" rather than "experiment" because, no matter how much theory goes into the design of an inquiry into a complex system consisting of many individual animals, the important conclusions most often involve phenomena unanticipated in the original design of the effort. Constructs will be underlined.

If mammals with fixed homesites, home range centers, were simple stimulus-response automata they would sequentially deplete resources from the homesite outward, thus requiring increasing energy expenditure over time. This trap has been avoided by the evolution of CNS control mechanisms that influence the probability of termination of behavioral states. No matter how long an outward trip from home has lasted, there is a constant probability that the excursion will terminate in the next unit of fixed time. While on the outward trip the animal behaves as if perceptually blind; only a very novel stimulus will interrupt the normal CNS control of trip termination. Once normally terminated some other behavioral state will begin. Which one it will be is usually by the chance operation of the several probabilities of initiation, a unique probability for each behavioral state.

The function of these two types of probabilities in two dimensional space make responses per unit area decrease with distance from the homesite. Since most resources, including the position of associates, are randomly distributed over space, the CNS probability functions controlling the inception and cessation of behavioral states evolve to apply to all behavioral states, even including sleep. Furthermore, since resources more distant from the homesite are utilized less frequently, neighboring individuals shift the sites of their homes closer together until the overlap of their ranges produces a uniform impact on all resources everywhere.

When this happens, every solitary animal will have contact with 18 associates. The evolutionary demand to increase predictability of perception, including knowledge of associates, leads some individuals to shift their homes towards a more dominant associate. Spatial and perceptual opportunities demand that the mean size of these loose clusters or constellations include 12 adults, the optimum group. From this state the evolutionary force of need for predictability of social knowings culminates in compact groups sharing a homesite. As groups of optimum size of 12 adults evolve, changes in heredity occur that

will alter behavior to optimize meaningful relations among associates. Under optimal conditions, 25 percent of inter-individual contacts are gratifying. The essence of the meaning of optimal group size is thus the optimization of gratification. However, the chance differences in receptivity to engaging in meaningful social relations, when two individuals meet, leads to some individuals gaining more satisfaction from contacts. Furthermore, the more an individual is frustrated in its relations the more its behavior becomes deviant, and the more it withdraws, both psychologically and physically. Members of the group thus become differentiated with respect to activity level, alertness, and propensity for engaging in social relations. Measures of these differences we term, social velocity.

When the members of an optimum-sized group are ranked by their social velocities it is found that the difference in velocity between any two adjacent ranked individuals is a constant, and that the absolute value of the minimum velocity individual is the same as this constant. Death often follows any persistence of velocity below this minimum. These differences in behavior in an optimum-sized group produce three clearly recognizable types. The 5 highest velocity individuals (42% of the total) form an alpha normal group. Most of the behavior promotes species survival. The next 4 lower velocity individuals (33% of the total) form a beta creative potential group. Many aspects of their behavior are quite deviant. One of their outstanding traits is that they keep probing into all aspects of their environment even though this regularly exposes them to aggressive retribution from dominant associates. They more readily take advantage of changed circumstances, and rats of this kind have been observed to engage in manipulations of their environment comparable to the discovery of the wheel by humans. Finally, there is a third gamma withdrawn group consisting of the three lowest velocity individuals. They are so withdrawn and deviant as to make little contribution to species survival. Were we dealing with humans, rather than rats and mice, we would expect this one-fourth of the population to become extremely psychologically or socially handicapped during some portions of their lives. All of the above is with reference to life in the best of all possible worlds, that is in the context of optimum-sized groups.

Increase in population density is usually accompanied by increase in group size. Under these circumstances four or five alpha and beta group members retain a modicum of their former behavior, whereas all the many others become gamma type withdrawn individuals. Even the alpha and beta type individuals undergo such drastic changes in behavior and physiology that their optimum group size is reduced to less than half of that dictated by their heredity. Inbreeding, by reducing flexibility of adaptation, also tends to reduce optimum group size to half that of the wild-type species. Furthermore, where members of a group are characterized by widely varying genotype, the size of the optimum group also becomes smaller.

When we turn to a population consisting only of groups of optimum size, a differentiation among them develops over several generations that is very similar to the differentiation of social velocity which develops among members of a group. A few groups will only contain alpha type individuals. Progressively more groups will contain more and more beta and gamma type individuals until at the extreme groups contain only gammas. This social class structuring of the population in the face of increasing population density increases chances of species survival by preserving reproduction in the high social class groups.

The closer a group lives to particularly preferred resources the more likely it will become of high social class.

There is a particular type of structure and symmetry of the habitat which reveals additional principles of spatial ordering of groups by social class. See Fig. 6. If the designed environment (a closed universe) has a perimeter approaching a circle in shape, and if subareas (cells) around the periphery are each structured to provide the requirements for a group of optimum size, then the following spatial class structuring emerges within 3 to 5 generations. Two adjoining groups will become highest class. The two groups on the opposite side of the habitat will become lowest class. If a line is drawn between these two pairs of groups, it will be noted that a gradient in social class develops from high to low in either direction around the habitat. Such more systematic structuring of groups provides even greater species survival chances despite increase in population density over generations.

Considerable intercommunication always occurs between members of neighboring groups. The above discussion of social class leads to the conclusion that a communication network characterized by bilateral symmetry with anterior dominance, in the sense of heightened efficiency of processing information, is of survival value. It follows that attributes of the physical environment which facilitate such communication networks also foster survival. This conclusion applies to the living space of groups as well as of a larger population. However there are other attributes of the physical environment which influence individual behavior, as well as group and population dynamics. Brief comment on the more important of these follows.

Compartmentalization: The CNS is only capable of 5 major types of behavioral states with respect to the probability functions which govern their initiation and termination. Mean time per day in these states increases in log steps from the shortest duration state of less than a minute to the longest one which lasts about three hours. Each behavioral state has two sub-types, one more acquisitory and the other more incorporative. This gives a total of ten behavioral states within which all variability in behavior and sleep must be encompassed. Stabilizing behavior in harmony with brain function is thus fostered by compartmentalizing the environment and structuring each compartment to be more favorable for the expression of one of the behavioral states.

Binary decision communications networks: Binary networks of routes of travel develop even in natural habitats lacking compartments or major barriers. Compartmentalization of the habitat makes it easier to design such travel networks. They are easily learned and increase the efficiency of behavior. Two derivative principles: (1) There should be at least two routes of travel from any specific point to each specific goal. (2) There should be at least two goals of the same type which can be reached from any point by a different route. Implementation of these principles increases choice and reduces inter-individual strife, while at the same time increasing a generalizing theory building capacity which fosters better coping.

Uncertainty and change: A population which is stable with reference to its numbers and environment accumulates errors faster than they can be corrected and will thus run a high risk of extinction. Even a static diversity of difference among cells of the environment produces sufficient system differ-

entiation and instability to encourage the maintenance of a high level of adaptive capacity. Temporal uncertainties of the outcome of responses encourage development of more complex adaptive strategies of behaving. Lastly environmental change diverts attention from associates and reduces the stress accompanying increase in density above the optimum.

These principles of environmental design were incorporated into the habitats of the current studies illustrated in Figures 5 to 7. Populations of animals will approach an "intrinsic" rate of growth (maximum biologically possible) when normal restraints of disease, predation, food shortage, and inclement weather are removed. Under these conditions, the intergeneration time span reduces to about one third of that normally occurring in nature. This means that onrushing input of new generations impedes the maturation of adult behavior. There arises an ultimate generation, by the time density reaches eight times optimum, for which behavioral maturation ceases near the age of weaning. These autistic-like subjects are incapable of any of the social behaviors essential for species behavior such as territorial defense, courting and mating. Members of the population age and die until population extinction ensues.

About 40 thousand years ago humans "discovered" a means for continually increasing the rate of population growth and still be able to realize the level of meaningful contacts per day consonant with that demanded by an heredity attuned to life within groups of 12 adults. Diversity of social roles was kept proportional to population density in physical space. The actual "discovery" was one of increasing the amount of information capable of being codified into concepts which are of utility for better obtaining needed resources, or for channeling contacts through individuals functioning in specific roles. The enlarged information base comprised a new kind of space, conceptual space, which preserved density constant despite actual increase in population size.

Through this self-propelling process, each successive doubling of human population size has required only half the time as for the prior doubling. Such an increasing rate of population increase is unique in the animal kingdom. Over such increase, the increase in conceptual capacity of individuals for adaptation has increased proportional to the square root of population size. However, this millennia-long pattern of population increase terminated in 1975. Beyond this date only theory can guide policy for human survival.

Figures 1 and 2 reflect the theory we have developed. There will be an initial transition period of 200 years involving a shift from a population of increasing size to one that continuously diminishes. It promises to be a period of potential value conflict and tension in human relations unparalleled during the evolution of culture. (See next page for Figures 1 and 2.)

If the repercussions of this transition are to be eased, the central trend of evolution must be preserved. That is to say, individual capacity for adaptive behavior must continue to increase. In the present case for humans, means for diffusing and interrelating ideas must be developed that will extend and partially replace the inter-human communication network that has previously enlarged as population has increased. Sleep by rats provided the clue to resolution of this problem. Rats have four sleep behavioral states. The shortest of these, with a mean duration of 27 minutes, we refer to as "day-

FIG. 1 Evolutionary World Population Model. (Human)

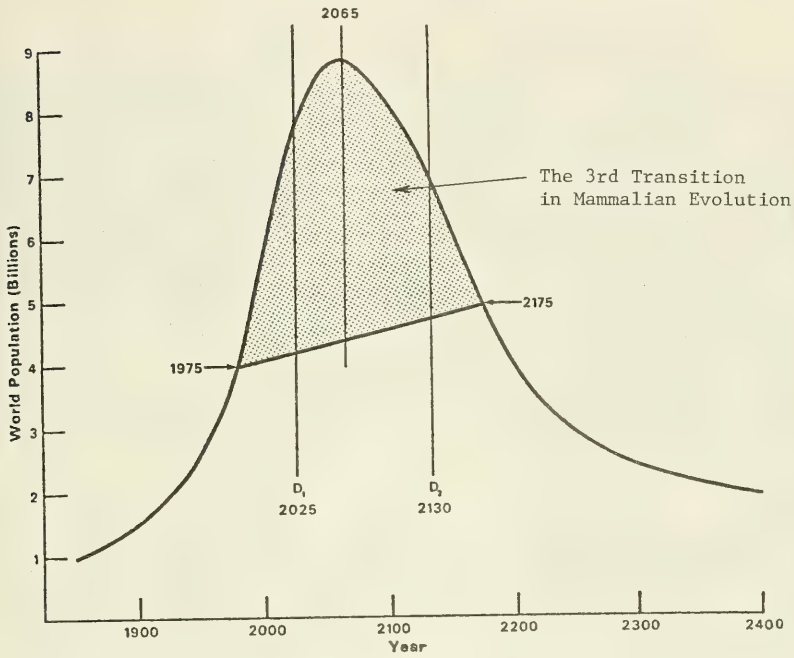
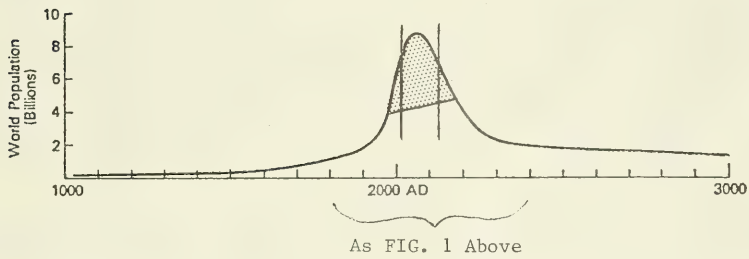


FIG. 2 Evolutionary Population Transition. (Human)



dreaming". It is characterized by rats lying prone in public space with eyes partially closed. They rarely express reaction to associates while in this state. The mean duration of this sleep is identical with that of sequences of motor behavioral states that intervene between any two episodes of sleep. Therefore dream sleep by rats seems to be neurologically identical with sequences of motor behavioral states, but lacks the motor component.

Study of 6000 paragraphs from literature pertaining to our research program revealed that they conformed to the same type of CNS control of duration as characterized motor behavioral states of rats, and that their aggregation into statements or sections of chapters resembled dream sleep by rats with respect to duration. We conclude that such written material represents an expression of thought behavioral states. From this insight a general theory has emerged as to how concepts are interrelated within the brain and how memory representations of thought behavioral states are recalled. Guided by this theory it has been possible to develop algorithms for computer simulation of brain function in reassembling thought behavioral states from many individuals into a coherent manuscript. Our first efforts in this direction suggest that this approach holds promise for developing into a more effective means for integrating ideas expressed by many individuals.

The above represents the minimal background to appreciate how our three major projects (one with mice, one with rats, and one on human population) relate to our broad objective. However, in the present report primary emphasis will be on the two animal model studies.

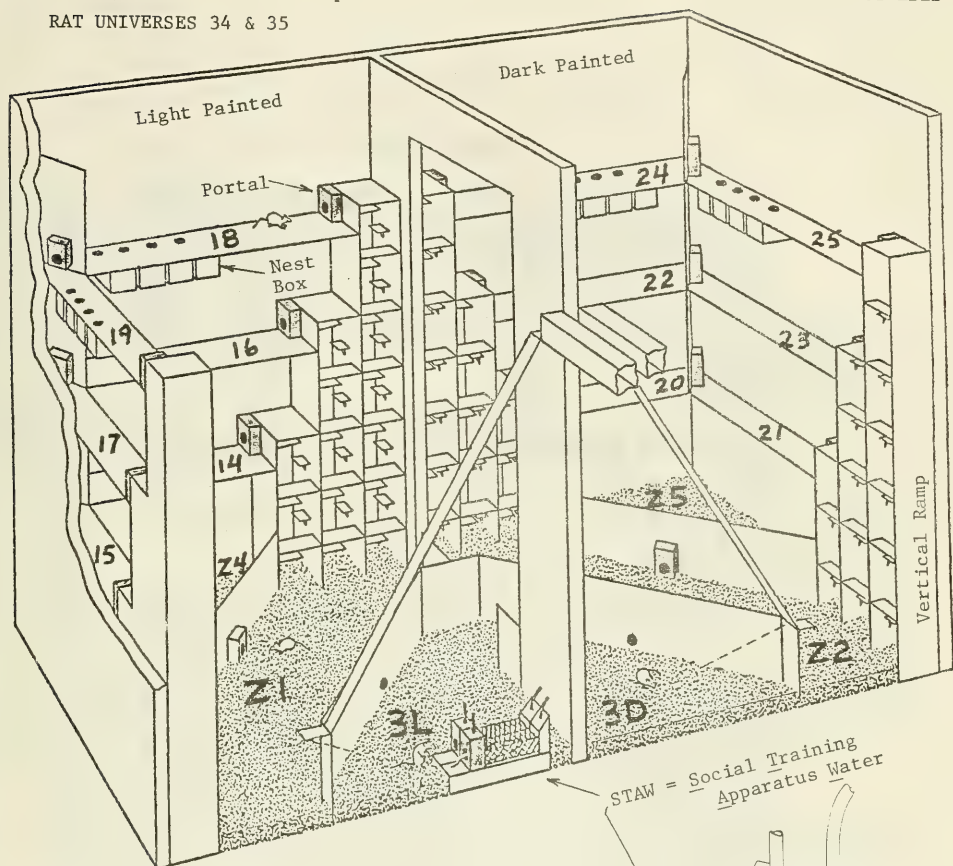
Methods:

Subjects: We increased genetic diversity, and therefore adaptive capacity as follows. The mouse population (Study 133) was initiated with F₁s of a cross between the Balb C and A inbred strains. The rat populations were initiated with the products of a 6-way cross of 4 domesticated breeds and 2 wild type strains. The mice were followed for an 8-generation period, 200 days per generation period. Progeny born toward the end of each period were allowed to survive in numbers sufficient to double the population size each generation. The two populations of rats were studied over two generations for 14 months of the adult life of each generation. The first generation of each population consisted of two optimum sized groups. The second generation was allowed to survive only after the first generation had reared many litters to weaning. First generation rats were removed when their second generation progeny were subadults. Second generation group sizes exceeded twice the optimum size.

Habitats: (Figs. 3 to 7) Bilateral symmetry forms the core aspect of the habitats for both rats and mice. The rat habitat (Figs. 3 to 5) provides identical structures for each of two groups. By the simple expedient of painting one side darker than the other, the dark painted side became the residence of the dominant (higher social class) group of these nocturnal animals. The mouse habitat (Figs. 6 and 7) provides space and structures forming "cells" for 16 groups. However, these 16 cells, by higher intervening barriers between every other cell, form bilaterally symmetrical units of adjacent pairs of cells (Fig. 7). The nest boxes of the clockwise cell of each pair were located at twice the elevation of those in the other cell. The resultant differential in energy expenditure ("difference in income") required to move between nest

FIG. 3 Main Aspects of Rat Habitat
RAT UNIVERSES 34 & 35

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EQUIPMENT NOT SHOWN

1. Clan Training food hoppers Z4, Z5
2. Dominance stand Z1, Z2
3. Nest material source for zones 14-25
4. Paired nest box access compartments

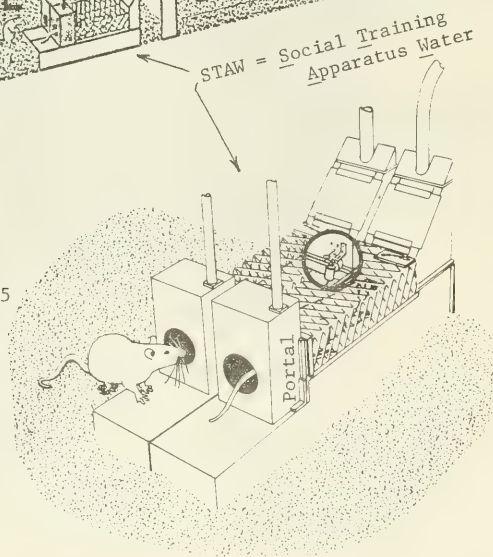


FIG. 4 Floor Plan of Rat Habitat

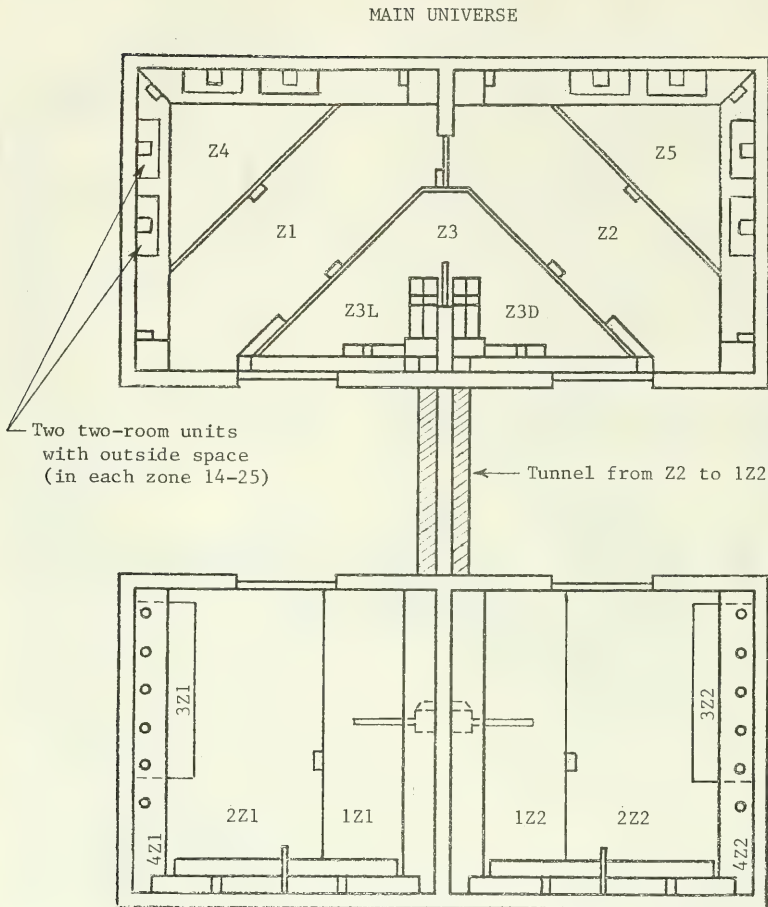


FIG. 5 Schematic Representation of Rat Habitat as a Bilaterally Symmetrical Communication Network

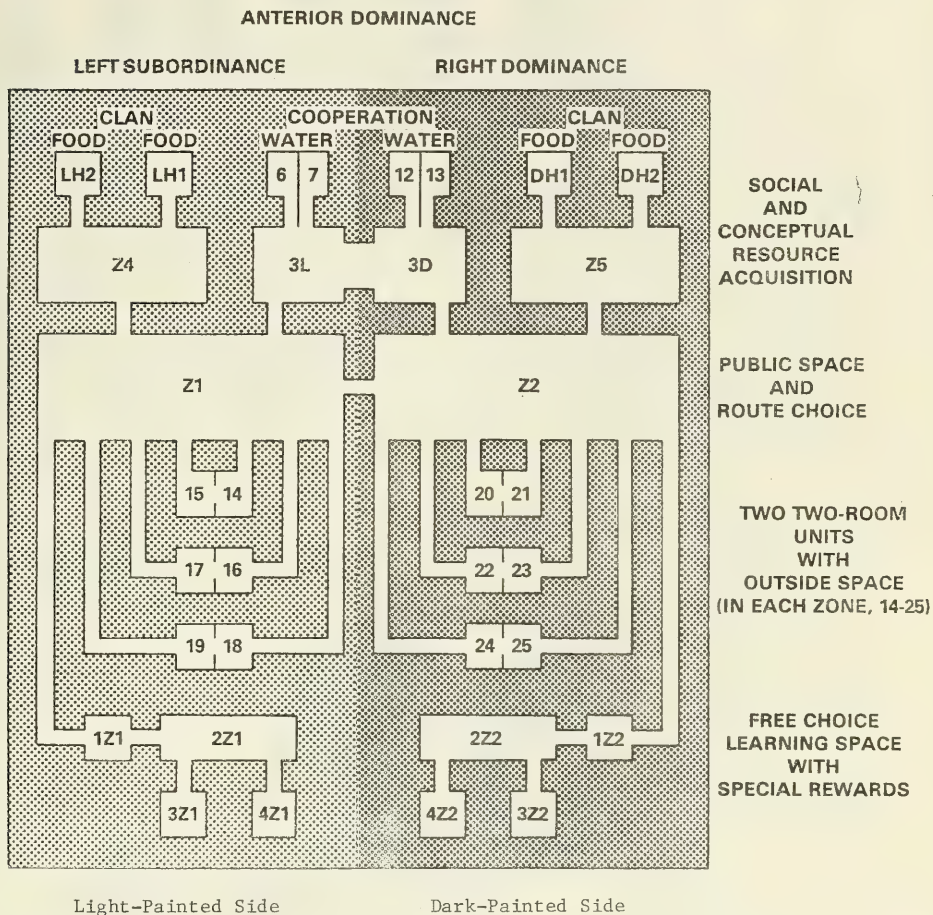


FIG. 6 Mouse Habitat

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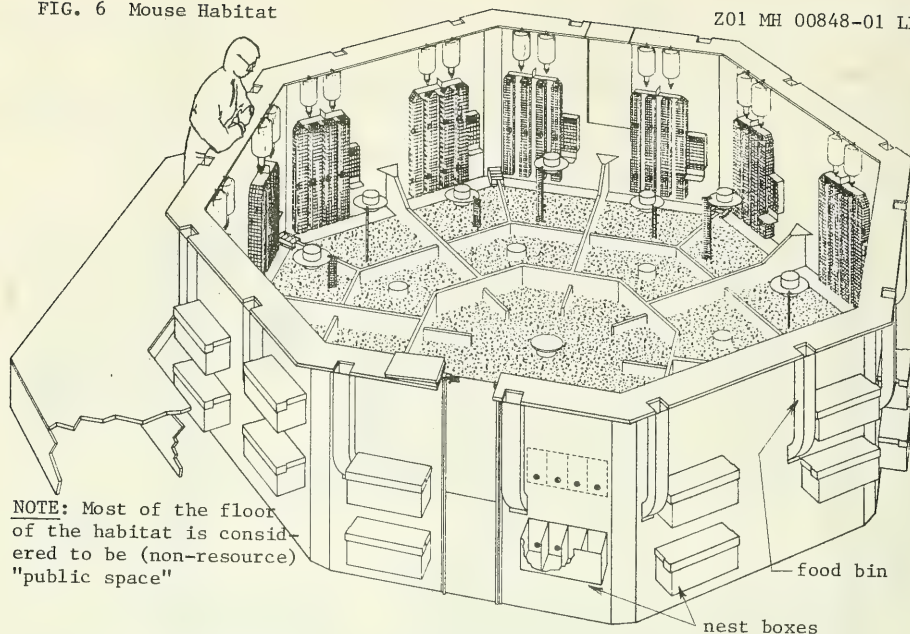
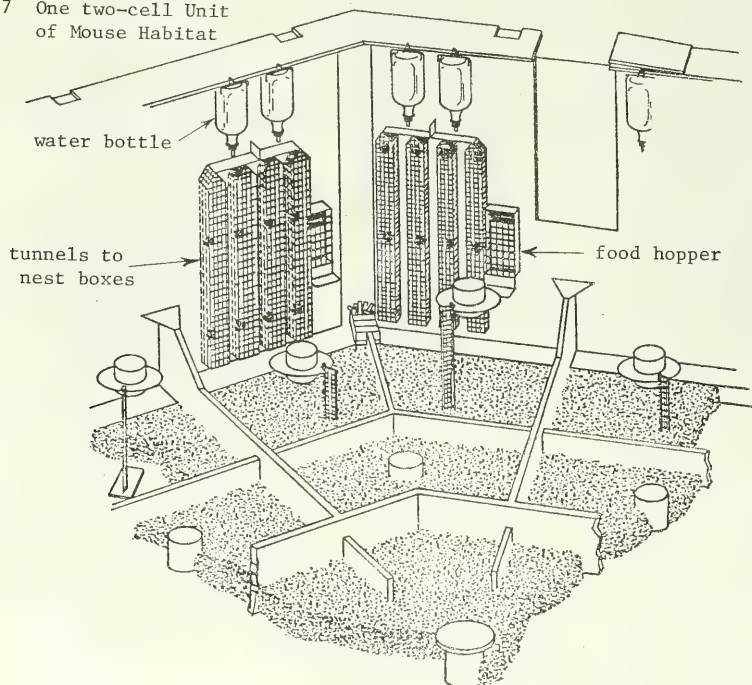


FIG. 7 One two-cell Unit of Mouse Habitat



boxes and resources available outside led to the cell with the lower nest boxes becoming the residence of a group dominant to its paired neighboring group. A radial symmetry (Fig. 6) was superimposed on the whole mouse habitat ("universe") by forming a near-circle out of 8 of these pie-shaped bilaterally symmetrical sets of 2 cells.

Rat Special Recording Devices: Every rat carried an implanted passive resonator. Whenever a rat passed through a "portal" passage between two adjacent compartments, its identity, location, direction of travel, and time of day were computer recorded. STAWs (Social Training Apparatus Water -- Fig. 3) provided the source for all water. All members of the experimental populations were exposed to STAWs set for the COOP condition, the requirement that a rat be on each side of the STAW for either to obtain water from pressing a lever. The portals to zones 2 and 4 (food compartments) provided information to the computer as to which rats were in a food compartment. Motorized doors over the feeding surfaces of the hoppers could be opened or closed by signals from the computer. During the last one-third of the lives of members of the experimental population, the members of the group living on each side of the habitat were divided into two groups, High vs Low Status (clans A & B). Access to food occurred only when members of one clan were present in a food compartment. Entry of a member of the other clan caused the door to close over the food hopper, and it would stay closed until all the members of one or the other clan departed. Three free-choice rewards could be obtained from recording devices in each of the two learning space rooms of each rat habitat. Gradually response requirements were increased to make it easier for the rewards to be obtained if two rats cooperated.

Visual Observations: Coded dye marks were placed on the fur of both mice and rats to provide individual identification at a distance. For rats, 4 two-hour periods of observation were made on each of 2 days, every 21 days for each of the two populations during their 2nd generation (Total: 760 2-hour periods). One detailed observation was made each 1.5 minutes. For mice, 5 80-minute periods of observation were made on 200 marked subjects for 10 days, twice during each of 7 200-day periods (i.e. 700 80-minute periods). Observations by subject identity were restructured to provide computer access relevant to 80 to 150 item behavior taxonomies. The exact location (one place out of several hundred in a habitat) was recorded for each observation of behavior.

Vocalization of Rats: Analog power-kHz vocalization input was transformed into digital format to represent small time slices. Concurrent dictations of rat behavior, as well as portal passage recordings permit associating vocalizations with behavior.

Surveys: Every subject was caught and examined periodically to determine resting time residence, health and reproductive status. Surveys were made each 21 days for rats, and every 21 to 42 days for mice. All data is computer accessible.

Data File Structuring: Considerable attention and effort has been devoted to make all data computer accessible in a way that we can compare subjects or groups across population history within a particular habitat, as well as between habitats regardless of whether the subjects were mice or rats. In this data file structuring we have made the computer tape conform suffi-

ciently to that of the more pilot-type studies of 1967 to 1973 so that we can go back into these earlier data files as required to make new types of analyses that are suggested by the recently completed studies. Our entire data base, including that related to human thought behavioral states, encompasses about 25 million items. Advances in computer technology, and analytical procedures over the past few years make it now quite practical to engage in the analysis and integration of the many variables affecting complex bio-behavioral systems such as we have been investigating.

Major Findings: In 1974 we set out to conduct three interrelated studies that we believed were crucial to provide insights about behavior in the context of group and population dynamics. The magnitude of this effort amounted to a factory operation requiring nearly all the 64 person years available to the Unit between 1974 and 1982. However, analyses of sample data each year, provide the basis for some major conclusions:

1. Population extinction cannot be avoided in mice after the population has continued for a one-generation time span at 8 times optimum density. However, it must be noted that the three factors of greater genetic diversity, improved physical environment, and longer inter-generation time did prevent the origin of an autistic-like state that in earlier studies was so severe as to preclude any reproduction. And although members of the terminal generation of the present study could not successfully rear young under conditions of excessive crowding, they recouped their behavior and successfully reared young when removed and placed in small groups; this regardless of the duration of their exposure to excessive crowding.
2. Providing rats with opportunity to learn cooperative social role behavior simulates the theoretical origin of human culture in that rats which have acquired such behavior can tolerate life at densities above biologically optimum levels. Members of the control population exhibited extreme intolerance of maintaining relationships across the spatial interface between groups. Controls persisted in preventing inter-group contact by plugging the portal passages between the two sides of the habitat. In contrast, the cooperating, experimental rats exhibited more symbolic threat behavior both to intra- and inter-group associates. We believe that it will be possible to develop quantitative indices of this generalized behavior of the cooperative rats, that they expressed even in the face of moderate crowding. The words relaxed, tolerant, empathic, altruistic and compassionate best convey the quality of overall behavior of the experimental rats derived from learning two cooperative tasks.
3. Thought Behavioral States (Humans) There appears to be a pre-linguistically evolved propensity of developing a 3-dimensional network within the brain of the relationships among 1625 affectively loaded basic, experientially derived, concepts. An indepth permutated concept associational index based on this theoretical construct has proved to be an extremely useful tool to associate paragraphs (thought behavioral states) with shared implications contained in a 2058 paragraph data base on important researchable problems focussed on adaptation, environment and population.

Significance to Biomedical Research and the Program of the Institute:

Our research indicates that there are evolved genetic requirements for humans, as well as many lower mammals, to experience the number of inter-individual contacts per day associated with life within an optimum group size of twelve adults. Certain genetic constitutions, or exposure to persisting crowded conditions of living, reduce the optimum group size, decrease tolerance to associations with others, and increases behavioral deviance. It follows from this type of research, that development of necessary knowledge to prevent the origin of such pathologies requires emphasis on how contact among individuals is influenced by environmental structure.

Our pursuit of animal studies in the context of their bearing on human population problems has culminated in a conclusion of even more importance to mental health and to survival of humanity. This is that we have just entered (1975) the third transition crises in the history of mammals. The first involved acquisition of the associated capacities for rearing very dependent young and for maintaining a fixed homesite. The second was the inception of culture which was associated with the ability to maintain meaningful contacts per day consistent with prior life in groups of 12 adults, even though population density increased. Our present 200 year transition from increasing to decreasing population will be accompanied by greater reliance on computer facilitated interrelating of ideas. Results from our research provide some of the beginning clues for developing principles of designing the physical, social and conceptual environments that will help us through this transition.

Proposed Course: Primary emphasis over the next year will be on the analysis of our two animal model studies. Our strategy will be to define a set of basics on which more complex analyses depend. They are:

1. Home range (resting time vs active hours) for all individuals during each generational period including shifts in place of residence.
2. Defining the membership of spatially localized groups during each generational period.
3. Determining social velocity of all subjects for whom adequate observations are available.
4. Correlating health, reproductive and behavioral variables with social velocity.
5. On the basis of No. 4, assign indices of social velocity to members lacking detailed visual observations.
6. Reevaluate the characteristics of local groups on the bases of the social velocity and behavioral characteristics of the group.
7. Determine how the above analyses contribute to understanding the changes in reproductive competence and behavioral deviation as the populations increase in size.

8. Conduct a detailed analysis of behavioral states by environmental compartment, time of day, and behavioral characteristics of subjects.

Publications: Calhoun, John B., Editor, Environment and Population: Problems of Adaptation. New York, N.Y., Praeger Publishing Co. 1982 (in press).

Honors (to Calhoun):

1. Six lectures given to the American Academy of Pediatrics, November 1981.
2. Selected for the 3rd Annual Award for "significant contributions toward understanding the family" to be presented November 1982 by the Georgetown University Medical School's Family Center.
3. Department of Health and Human Services, ADAMHA Administrator's Award for Meritorious Achievement "For innovative research on the effect of environment on behavior, and the impacts on animal and human populations". (Award ceremony July 31, 1982; final confirmation July 14, 1982.)

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00881-26 LCM |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Intermediary Energy Metabolism in Mammalian Brain | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: E. E. Kaufman OTHER: T. Nelson L. Sokoloff T. Duffy G. Crosby | Research Chemist Staff Fellow Chief, Lab. of Cerebral Metabolism IPA and Professor, Depts. Neurology and Biochem., Cornell Univ. Medical College, NYC Guest Worker | LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Cerebral Metabolism | | |
| SECTION Section on Developmental Neurochemistry | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 4.25 | PROFESSIONAL: 3.50 | OTHER: 0.75 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK This report covers three projects which are currently in progress. The major project is a study of the origin, mode of action, and metabolism of <u>γ-hydroxybutyrate</u> (GHB). Biological factors which regulate the enzyme that converts GHB to succinic semialdehyde (SSA) have been determined. An antibody to this enzyme is being used to establish its identity with <u>D-glucuronate reductase</u> . Several compounds such as putrescine, arginine, and <u>β-hydroxybutyrate</u> have been shown to increase GHB levels. Present studies are directed toward determining the preferred <u>precursor</u> . A second area of study concerns the effect of elevated levels of <u>acetoacetate</u> on the formation of <u>GTP</u> . A good inverse correlation between blood <u>acetoacetate</u> and <u>brain cyclic GMP</u> has been established and suggests that GTP synthesis may be decreased in the mitochondria in disease states such as diabetes in which severe ketosis can occur. Experiments are in progress to measure the effect of ketosis on mitochondrial GTP formation in the brain. A third project nearing completion has demonstrated that <u>2-deoxy-D-glucose</u> can be incorporated into <u>glycogen</u> . Measurements of the amount of 2-deoxy-D-glucose which is incorporated into brain glycogen in 45 minutes indicates that it comprises 1-2% of the radioactive material in brain. | | |

Project Description:

This project continues into its third year of studies on the origin, mode of action, and metabolism of GHB, a compound which is a normal constituent of mammalian brain, and which may function as a neuromodulator or possibly as a neurotransmitter. When administered in pharmacological doses, GHB produces a flattening of the EEG, a reversible trance-like state, and a profound depression of cerebral glucose utilization. This compound is being used clinically in Europe as an anesthetic adjuvant. Preliminary trials indicate that it is effective in the treatment of narcolepsy and may be of use in the treatment of stroke. Recently it has been reported that GHB can block the myoclonic jerks produced by injecting mice with muscimol. This suggests that GHB may be of value in the treatment of Lance-Adams Syndrome and other forms of myoclonic seizures. Because GHB is a compound which is used clinically, and because it is a naturally-occurring compound, we feel that it is important to understand not only pathways of metabolism of GHB, but also the factors which control these pathways.

An enzyme capable of metabolizing GHB has been found in tissues such as liver and kidney as well as in brain. This enzyme, an NADP^+ -linked alcohol oxidoreductase which interconverts GHB and SSA, has been purified from hamster liver and partially purified from hamster brain. Throughout this report this enzyme will be referred to as GHB dehydrogenase so that the catalytic activity of interest to this laboratory will be clear. Studies completed on GHB dehydrogenase include substrate specificity, enzyme kinetics, molecular weight determination, and inhibition studies with the anticonvulsant agents, diphenylhydantoin, amobarbital, and valproate.

This project is directed at understanding the role of naturally-occurring GHB as well as the metabolic pathways involved in its synthesis and degradation. Since the compound is also used as a drug, other important questions arise concerning its pharmacological actions. In addition to the pharmacological effects already mentioned (flattening of the EEG, trance-like state, and depression of cerebral glucose utilization) it has recently been reported that GHB increases the survival of animals which have been made hypoxic. The authors report that the blood and brain tissues of animals which have received GHB showed marked changes in the levels of some intermediates in carbohydrate metabolism as well as changes in levels of high energy phosphate compounds. These studies have led to the proposal that GHB may be useful in the treatment of stroke.

We have recently found that GHB dehydrogenase also catalyzes the reduction of D-glucuronate to L-gulonate. The reduction of D-glucuronate to L-gulonate is an important step in the pathway leading from UDP-glucose to L-ascorbate or to L-xylulose and the pentose pathway. We have recently investigated the effect of both L-gulonate and of D-glucuronate on the rate of oxidation of GHB. In the presence of limiting concentrations of NADP^+ or with limiting concentrations of NADP^+ and inhibitory concentrations of NADPH , D-glucuronate can increase the rate of oxidation of GHB by up to eightfold. Since GHB dehydrogenase is a soluble enzyme found in the cytosol and since the cytosol of most tissues contains low concentrations of NADP^+ as well as relatively high concentrations of NADPH , D-glucuronate may be an important factor in controlling GHB metabolism in vivo.

The physical and chemical properties of GHB dehydrogenase have been investigated. Treatment of GHB dehydrogenase with a reducing agent such as

dithiothreitol produces marked inhibition of activity. This inhibition can be at least partially reversed by the addition of an oxidizing agent such as hydrogen peroxide. We have recently found that complete reversal of the inhibition by dithiothreitol can be achieved by the addition of oxidized glutathione. This suggests that the activity of GHB dehydrogenase may be regulated by the ratio of oxidized to reduced glutathione.

A study on the purification and characterization of GHB dehydrogenase has been completed and published. This enzyme has been purified 300-fold and found to exhibit a single band on gel electrophoresis. The protein is a monomer with a molecular weight of $\sim 31,000$ daltons. GHB dehydrogenase is inhibited by two anticonvulsant agents, amobarbital and diphenylhydantoin, as well as by valproate, a drug used in the treatment of epilepsy. Pyrazole, the specific inhibitor of NAD⁺-linked alcohol dehydrogenase (ADH), does not inhibit this enzyme. Diethyl-dithiocarbamate, the reduced form of the drug antabuse, as well as KCN, both metal-chelating agents, inhibit GHB dehydrogenase. This suggests that the enzyme may contain either copper or zinc as an integral part of the protein. This possibility is currently being investigated.

We now have evidence that GHB dehydrogenase is identical to D-glucuronate reductase and that the ability to oxidize GHB to SSA represents a new activity for D-glucuronate reductase. The unusual attribute of GHB dehydrogenase is its ability to couple these two reactions (the reduction of D-glucuronate to L-gulonate and the oxidation of GHB to SSA) and in so doing to alter markedly the kinetic constants for GHB, and the cofactors, NADP⁺ and NADPH. A detailed study of the kinetic constants in both the coupled reaction and in the uncoupled reaction has been completed. This study showed that the kinetics of the uncoupled reaction proceeded by a Rapid Equilibrium Random BiBi mechanism. The alteration of the kinetic constants for GHB and the cofactors, NADP⁺ and NADPH, brought about by coupling the oxidation of GHB to the reduction of D-glucuronate are of sufficient magnitude to make it possible to propose that this reaction may proceed under conditions approaching those which exist in the whole tissue. This is of significance since the catabolism of GHB has been shown to proceed through SSA to succinate and the citric acid cycle. In this pathway, the oxidation of GHB to SSA would be the first step. Any acceleration or inhibition of this reaction would be reflected in altered tissue levels of GHB.

The effect of a number of biological intermediates on the oxidation of GHB has been studied. The citric acid cycle intermediates succinate, pyruvate, α -ketoglutarate and oxaloacetate, the ketone bodies, acetoacetate and β -hydroxybutyrate, and the products arising from the transamination of phenylalanine such as phenylacetate were all inhibitory to varying degrees. The α -keto acids, α -ketoglutarate and oxaloacetate, were among the most inhibitory. However, when the α -keto group was replaced by an α -amino group the resulting amino acids, L-aspartate and L-glutamate, either did not inhibit or produced a slight stimulation as did L-alanine and GABA.

A detailed study of the effect of pH on the kinetics of both the simple (uncoupled) oxidizing GHB to SSA and on the coupled (+ D-glucuronate) oxidation of GHB has been carried out. Both V_{\max} and K_m were found to vary with pH. This resulted in a marked change in the pH optimum for this reaction when the velocity was measured at low concentrations of GHB rather than under V_{\max} conditions. As the concentration of GHB approached physiological levels the pH optimum shifted

from pH 9 (V_{\max} conditions) to a more physiological pH (7.0 for the coupled reaction at 0.5 mM GHB and 7.5 for the uncoupled reaction at 1.0 mM GHB).

Alcohol dehydrogenase (ADH) is also capable of catalyzing the interconversion of GHB and SSA in vitro. The K_m for GHB with ADH is, however, an order of magnitude higher than the K_m with the GHB dehydrogenase. In vivo studies on the half-life of [^{14}C]GHB (in plasma) in the presence and absence of various inhibitors have been carried out in an effort to assess the contribution of these two enzymes to the metabolism of GHB in the whole animal. Results of these studies show that pyrazole, a potent inhibitor of ADH, does not affect the half-life of GHB. The same concentration of pyrazole produced severe inhibition of ethanol metabolism in the whole animal. This suggests that ADH is probably not the enzyme responsible for the oxidation of GHB in the whole animal. Preliminary results show that two compounds which inhibit the oxidation of GHB catalyzed by the NADP^+ -linked oxidoreductase, dilantin and L-gulonate, increase the half-life of GHB in the plasma (indicating a decreased rate of metabolism). These results lend support to a role for the NADP^+ -linked GHB dehydrogenase in the metabolism of GHB in the whole animal. Recent evidence suggests that GHB dehydrogenase is not involved in the synthesis of GHB but rather in its degradation.

Antibody. The purified NADP^+ -linked GHB dehydrogenase, which interconverts GHB and SSA, has been used to raise antibody in New Zealand white rabbits. This antibody will be purified and used to confirm the role of the NADP^+ -dependent GHB dehydrogenase as a degradative enzyme for GHB. The antibody will also be used to determine whether the GHB dehydrogenase from brain and extraneural tissue is indeed the same enzyme.

This antibody is being used as a probe in the study of the different oxidoreductases involved in the synthesis and degradation of GHB. In addition to studies with specific inhibitors and substrates, it should be useful in answering questions concerning the subcellular localization of the biosynthetic as well as the degradative enzymes. These studies are now in progress.

GHB levels in organs. The survey of rat organs for GHB reveals that GHB is widely distributed throughout all the organs which have been examined; its concentration in rat organs is shown in the accompanying table. Concentrations are expressed in nanomoles per gram of tissue.

| ORGAN | BRAIN | HEART | KIDNEY | LIVER | LUNG | MUSCLE | BROWN FAT | WHITE FAT | BLOOD |
|------------|-------|-------|--------|-------|------|--------|-----------|-----------|-------|
| Mean GHB | 2.29 | 12.4 | 28.4 | 1.4 | 1.5 | 10.2 | 37.4 | 0.42 | 0.55 |
| Std. Error | 0.13 | 1.9 | 4.6 | 0.3 | 0.2 | 1.6 | 2.1 | 0.27 | 0.27 |
| Number | 36 | 36 | 36 | 36 | 36 | 36 | 100 | 7 | 12 |

These findings suggest that GHB plays some role in many, if not all, of the organs in the body. Until those results were obtained, it had been assumed that the most likely function of GHB was to modulate dopaminergic neuronal activity. Now additional functions will have to be sought for GHB in non-neural tissues, and these new functions may also have relevance to the brain. The most likely precursors for GHB were thought to be glutamic acid, γ -aminobutyric acid, and SSA, since experimental evidence shows that each of these molecules is capable of contributing carbon atoms to the skeleton of GHB. The finding of high levels of

GHB in tissues such as kidney, which lack the means of decarboxylating glutamate to form γ -aminobutyrate, suggests that there must be other sources for GABA formation than glutamic acid. The most likely precursor may be putrescine or one of its metabolites.

Spectrophotometric Assay.

A new spectrophotometric assay for the detection of GHB has been devised. It is based on the use of the purified NADP⁺-linked GHB dehydrogenase and the synthetic co-factor, 3-acetylpyridine-adenine dinucleotide phosphate (APTPN⁺). The substitution of the synthetic co-factor shifts the equilibrium of the reaction $\text{GHB} + \text{APTPN}^+ \rightarrow \text{SSA} + \text{APTPN}^+ + \text{H}^+$ far to the right so that the GHB may be estimated by the amount of reduced co-factor which has been produced. We anticipate that this assay will prove useful in the estimation of blood and tissue levels of GHB.

Developmental Study.

A study has been completed in which we have determined both GHB levels and soluble cytosol NADP⁺-linked GHB dehydrogenase activity in several organs of developing rats from the late fetal stage to 20 postnatal days. GHB concentrations in the newborn rat brains and livers are two to three times higher than they are in the adult. The concentration of GHB gradually decreases over the first 20 days of life to adult levels. GHB levels in the kidney rise from a lower level in the newborn period to attain the high level characteristic of the adult by 20 days. The enzymatic activities, on the other hand, tend to increase from the time of birth to 20 days in all the tissues, including brain.

This pattern of developmental changes is of special significance since previous widely accepted studies by other workers have suggested that GABA is the precursor for GHB in the brain. A soluble cytoplasmic enzyme has been implicated in the reduction of SSA in the cytosol to form GHB. It is known that during the same period of development in the rat both GABA transaminase and glutamate decarboxylase activity are low at birth and increase by some tenfold. These findings are consistent with a rate of GABA production which is low in the newborn and increases with age, while our present study shows GHB, a putative metabolic derivative of GABA, is highest at a time when GABA synthesis is low and falls as the rate of GABA synthesis increases. These findings suggest that GHB formation in the perinatal brain may involve precursors other than glutamate and possibly other than GABA.

Precursor for GHB. One of the new aspects of this project is an effort to work out the biosynthetic pathway for GHB. We have used three main approaches to this problem. In the first, a number of carbohydrate intermediates, amino acids, and fatty acids were tested in an in vitro system for the ability to produce net synthesis of GHB. This has been a general survey of a number of possible precursors for GHB.

The second approach was similar to the first except that radioactive compounds were used in order to determine whether the compounds which gave rise to GHB in the first part actually contribute carbon atoms to the GHB molecule.

The third approach will be in vivo experiments in which a radioactive precursor is injected and GHB is then isolated from the various tissues to determine whether the putative precursor has been incorporated in the GHB pool.

Results from the general survey of precursors, and preliminary work with radioactive compounds (*in vitro*), indicate that D,L- β -hydroxybutyrate and citrate are both capable of stimulating the formation of GHB, and of contributing carbon atoms to the skeleton of GHB. However, in the crude system of cell homogenate there is such a large amount of endogenous precursor or precursors that the specific activity of the GHB is reduced to about 10% of the specific activity of the precursor. The endogenous precursor is found in high concentration (up to 1 mMol/gr of kidney) in the soluble fraction of mitochondria from fed rats. Such high concentrations of endogenous precursor make it unlikely that β -hydroxybutyrate or citrate serve as the major precursor. While β -hydroxybutyrate does not appear to be a major precursor, its stimulating effect on the synthesis of GHB from endogenous precursors in the soluble mitochondrial fraction is quite marked. In the presence of NAD^+ a concentration-dependence existed between D,L- β -hydroxybutyrate and GHB formation. Substitution of NADP^+ as the cofactor resulted in large amounts of endogenous substrate being converted to GHB even in the absence of D,L- β -hydroxybutyrate, though the inclusion of D,L- β -hydroxybutyrate resulted in even greater synthesis of GHB.

We infer from these results that there is a large pool of precursor which must be converted by an NADP^+ -linked enzyme to an intermediate. This, in turn, can be converted to GHB by an oxidative step which ultimately leads to SSA. The final reductive step would utilize the NADPH (generated at the NADP^+ -linked oxidation) or the NADH which could be generated in the presence of D,L- β -hydroxybutyrate. These results could be explained if putrescine is serving as the precursor. While putrescine added to kidney homogenate does not stimulate GHB synthesis, putrescine in the presence of an acetylating system does result in GHB synthesis. It is known that N-acetyl putrescine can be metabolized to GABA. The steps involve the oxidation of N-acetyl putrescine to N-acetyl γ -aminobutyraldehyde by a monoamine oxidase, and the N-acetyl γ -aminobutyraldehyde is further oxidized to N-acetyl GABA by an oxidoreductase. This oxidoreductase in rat kidney appears to be NADP^+ -dependent, though a similar dehydrogenase in *Pseudomonas* requires NAD^+ . NADP^+ would allow the N-acetyl γ -aminobutyraldehyde to be oxidized to N-acetyl GABA. After deacylation to form GABA the resulting SSA would be reduced to GHB in the presence of the NADPH which had been generated at the oxidative step. The stimulatory effect of D,L- β -hydroxybutyrate, and citrate (other than that deriving from their conversion to GHB), and that of other compounds such as ethanol, probably results from the generation of NADH during their oxidation. This NADH would then allow preexisting SSA derived from GABA and N-acetyl GABA to be reduced to GHB, though it does not permit the formation of SSA from N-acetyl butyraldehyde.

The kidney appears to be able to use arginine as a precursor for GHB synthesis (as judged by the net synthesis of GHB which is dependent on arginine). An acetylating system inhibits arginine's stimulatory effect upon GHB synthesis, thus suggesting that arginine may not be converted to putrescine in this pathway. Prokaryotic organisms are capable of converting arginine to GABA in a series of reactions which do not lead to putrescine. It may be that these enzymes are active in mammalian kidney and allow a similar conversion of arginine to GABA.

Thus far it appears that the major portion of GHB is derived from GABA. In brain GABA is formed from glutamate through the GABA shunt pathway; in other tissues preliminary evidence suggests that polyamines or arginine may serve as the GABA precursor. The synthesis thus may be linked to the citric acid cycle,

the urea cycle, and to the polyamines which appear to play some regulatory function within the cell nucleus. The degradation of GHB is most likely accomplished by glucuronate reductase under circumstances which require a stoichiometric oxidation of aldehydes such as D-glucuronate to occur. Furthermore, the degradation of GHB appears to be controlled by several naturally-occurring α -keto acids, and phenyl ethyl alcohols which have recently been shown to inhibit D-glucuronate reductase. Pharmacological levels of GHB have been shown in this laboratory to produce a striking reduction of glucose utilization by the brain. Taken together these findings suggest that GHB might serve as some form of intracellular messenger, perhaps linking both protein and carbohydrate metabolism with nuclear events.

Effect of Naloxone on the Pharmacological Action of GHB.

It has been reported that naloxone can reverse some of the pharmacological effects of GHB, i.e., the effect on EEG charges, the accumulation of dopamine in the nigrostriatal pathway and the behavioral effect. Investigations in this laboratory of naloxone as an antagonist of GHB revealed that the effect of GHB on cerebral glucose utilization could be partially reversed in selected regions of the central nervous system. It was also observed that naloxone affected the drop in body temperature caused by a pharmacological dose of GHB. This aspect of the GHB project has been completed and a report is being submitted for publication.

Proposed Course.

This project will continue on the course which has been outlined. It will include: 1) further kinetic studies with GHB dehydrogenase; 2) determination of the presence or absence of metal in this enzyme; 3) *in vivo* studies to determine the effect of various drugs, normal metabolites and alterations in physiological state on the metabolism of GHB; 4) investigation of the interaction of GHB with other pathways of carbohydrate metabolism; 5) developmental studies of GHB and of GHB dehydrogenase in brain and other tissues; 6) study of the biosynthetic pathway for GHB. If putrescine and arginine are the precursors of GHB it will be important to determine whether GHB has an effect on protein synthesis.

Project #2. Effect of Ketones on Cerebral Metabolism.

This laboratory has had a continuing interest in ketone body metabolism in normal as well as in pathological states. D- β -Hydroxybutyric acid dehydrogenase catalyzes the reversible interconversion between D- β -hydroxybutyrate and acetoacetate, the two ketone bodies found primarily in the liver when fatty acid utilization is increased, as, for example, in diabetes, starvation, high-fat diet, and various states leading to ketosis. Acetoacetate is the primary ketone body formed from fatty acid catabolism in liver and muscle, and it is converted to D- β -hydroxybutyrate by the action of β -hydroxybutyrate dehydrogenase. Once formed, however, D- β -hydroxybutyrate cannot be used directly in any of the tissues; it must first be converted back to acetoacetate by the action of the same enzyme. This raises the interesting question of what role this enzyme plays. Why does the body not utilize the directly-formed acetoacetate rather than convert it first to D- β -hydroxybutyrate only to oxidize it back to acetoacetate again to utilize it? There is evidence in the literature to suggest that excessive concentrations of acetoacetate in the tissues may be toxic, particularly in brain which has recently been shown to utilize ketone bodies in direct proportion to their levels in the blood. The possibility exists that the role of the enzyme is

protective, namely, to keep the acetoacetate concentrations low by storing it, in a sense, as D- β -hydroxybutyrate. It is noteworthy that there are several metabolic conditions characterized by ketosis and coma. Diabetic coma is one example, and recently Reye's Syndrome has been described. This is a frequently fatal disease of childhood which occasionally follows a viral infection, particularly influenza. It is characterized by fatty degeneration of the liver, ketosis, and coma.

Examination of the metabolic pathways and the enzymes responsible for the metabolism of ketone bodies in brain suggests possible mechanisms by which forced excessive use of ketone bodies in brain may lead to depressed cerebral functional activity. The ketone bodies enter the Krebs Cycle after their conversion to acetylCoA, which in the presence of succinyl CoA-acetoacetate CoA transferase reacts with succinyl CoA to form acetoacetyl CoA. For every acetoacetate molecule thus metabolized, there is one succinyl CoA degraded. This reaction thus competes with the reaction catalyzed by succinic thiokinase which synthesizes a molecule of GTP for every molecule of succinyl CoA which is converted to succinate. This diversion of succinyl CoA would be expected to deplete mitochondrial GTP, which in turn might be expected to interfere with cyclic GMP synthesis. There is now a considerable body of evidence that cyclic GMP is the second messenger in the action of the neurotransmitter, acetylcholine. Its depletion can be expected to result in depression of cholinergic functions in brain and lead possibly to depressed functional activity and even coma. The possibility that ketosis may lead to coma by depletion of brain cyclic GMP is currently being studied in this laboratory. A radioimmune assay for cyclic GMP has been established and preliminary results do indeed suggest that in diabetic acidosis there is a depression of cyclic GMP in brain.

During the past year this project has received considerable attention. Biochemical studies have been carried out on the brain and blood of four groups of animals (adult male Sprague-Dawley rats): control animals, diabetic animals (diabetes induced by streptozotocin injection), diabetic animals which received insulin, and fasted animals. These animals were monitored for blood glucose and ketone bodies as well as active physiological and biochemical parameters. Brain levels of phosphocreatine, ATP, GTP, GDP, and cyclic GMP were measured and correlated with blood levels of acetoacetate, β -hydroxybutyrate, lactate and glucose. In the diabetic animals there was good correlation between the blood level of total ketones and C-GMP in brain ($P = 0.002$) and also between blood β -hydroxybutyrate and C-GMP in brain ($p = 0.0024$) in spite of the fact that the GTP levels in the control and diabetic animals were almost identical. This suggests that there may be some compartmentation of GTP.

Projected Course.

We plan to examine GTP and other metabolites in subcellular fractions of brain following infusion of ketone bodies.

Project #3. Incorporation of 2-Deoxy-D-glucose into Glycogen.

During the past year published reports indicated that ^{14}C - and ^3H -labeled 2-deoxy-D-glucose are found in close association with typical glycogen granules seen on electron micrographs. This finding suggests that some of the 2-deoxy-D-glucose-6-phosphate is being further metabolized to glycogen, and indeed reports now claim that perhaps up to 10% of the 2-deoxy-D-glucose administered to the

mouse, the leech and the snail may be converted to glycogen. None of the reports contained vigorous proof that the label which appeared to be incorporated into glycogen was actually present as deoxyglucosyl units, rather than as a deoxy-D-glucose-6-phosphate or 2-deoxy-D-glucose adsorbed onto the surface of the glycogen.

Experiments performed in this laboratory during the past year confirm that 2-deoxy-D-glucose is incorporated into glycogen as glucosyl units. It was shown further that a significant amount of 2-deoxy-D-glucose phosphate (30-60%) could be released from the glycogen fraction after it had been prepared by standard methods. Proof that 2-deoxy-D-glucose could form glycogen was obtained by demonstrating that glycogen which had been stripped of adsorbed phosphates by passage over a Dowex 1 column and exhaustive dialysis could be degraded quantitatively by amylo-1,-4, 1-6-glucosidase to a substance which could pass through an Amicon PM-10 filter membrane. Treatment of the glycogen with phosphorylase "a" released 78% of the label in the form of a compound which did not migrate with 2-deoxy-D-glucose on thin layer chromatography, and which upon mild acid hydrolysis at 80°C migrated in the same spot as 2-deoxy-D-glucose.

It has been shown that under the usual conditions in which glucose metabolism is measured by the 2-deoxy-D-glucose method, no more than 2% of the radioactive material will be present as glycogen. The further metabolism of 2-deoxy-D-glucose-6-phosphate to glycogen is of no significance to the validity of conclusions which can be drawn from the results of the method as it was originally described since the ^{14}C label trapped at any step beyond the phosphorylation step by hexokinase will represent glucose which has been metabolized.

If an attempt is made to apply the 2-deoxy-D-glucose method to problems which require that labeled tissues be rinsed in water containing solvents, then much of the 2-deoxy-D-glucose-6-phosphate will be lost, leaving some or most of the less soluble 2-deoxy-D-glucose labeled glycogen. Thus the label no longer is directly related to cellular glucose metabolism, but is also related to the individual cell's ability to synthesize glycogen. Moreover, since brains which are to be used for histological examination are usually carefully dissected from the skull, the variable delay between death and freezing may be accompanied by unpredictable losses of glycogen since this macromolecule is rapidly phosphorylated after death.

Proposed Course.

This project has been terminated, and the results will be published.

Significance to Biomedical Research and Program of Institute.

Energy metabolism of the central nervous system has been the dominant interest of this Section since its establishment in 1953 when the Intramural Research Program was first organized. The studies described in this project are continued investigations along paths opened by previous research of the Section. The metabolic effects of γ -hydroxybutyrate were discovered here, and the present studies are designed to uncover the mechanism of its actions and, because it is normally present in brain, to elucidate its normal functions. It has been implicated in control of dopaminergic functions, in seizure states, and in anesthesia. The clarification of its origin, disposition, and function should serve to enlarge our knowledge of normal and abnormal functions and biochemistry in the brain.

Similarly, the first evidence that ketone bodies may play a role in brain energy metabolism was discovered here in this Section. It was subsequently shown by others that ketone bodies may substitute for glucose as a substrate for cerebral oxidative metabolism. There is, however, evidence that prolonged ketotic states may alter brain function. Diabetic keto-acidosis, for example, produces coma. Ketogenic diets diminish epileptic seizures in juvenile epilepsy. The present studies are directed at elucidating the mechanism of these effects and may lead to better understanding and treatment of epileptic states and some types of coma.

PUBLICATIONS:

Nelson, T., Kaufman, E., Kline, J., Sokoloff, L.: The extraneural distribution of gamma hydroxybutyrate. J. Neurochem. 37: 1345-1348, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00882-15 LCM | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | |
| TITLE OF PROJECT (80 characters or less) Studies on Regional Cerebral Circulation and Metabolism | | | |
| PI: OTHER: | L. Sokoloff C. Kennedy M. Kadekaro C.B. Smith M. Ito D. Dow-Edwards L. Porrino F. Orzi A.C. Tannenbaum H. Namba J. Jehle M. Ingvar R.B. McFarlin C. Goochee P. Gross P. Maeder | Chief, Lab. of Cerebral Metabolism Guest Worker Visiting Scientist Staff Fellow Visiting Associate Staff Fellow Staff Fellow Visiting Fellow Research Biologist Visiting Fellow Lab. Technician (Microbiol.) Visiting Fellow Computer Programmer Computer Programmer Visiting Fellow Visiting Fellow | LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH LCM NIMH |
| COOPERATING UNITS (if any) T.Duffy, Cornell U., NYC; M.Mishkin, Lab. of Neuropsychology, NIMH; J.C.Gillin, Lab. of Clin. Psychopharmacology, DSMR, NIMH; R.Kessler, Nuclear Med., CC, NIH; G.DiChiro, NINCDS, NIH; S.Rapoport, Lab. of Neurobiology, NIA, NIH; J.Saavedra, Lab. of Clinical Sci., NIMH; M.S.Buchsbaum, Biol.Psychiatry, NIMH. | | | |
| LAB/BRANCH Laboratory of Cerebral Metabolism, DBBR, NIMH | | | |
| SECTION Section on Developmental Neurochemistry | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland | | | |
| TOTAL MANYEARS: 12.0 | PROFESSIONAL: 7.0 | OTHER: 5.0 | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) A method has been developed for the quantitative determination of the rates of <u>local glucose consumption</u> in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [¹⁴ C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [¹⁴ C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping <u>metabolically</u> , and therefore functionally, linked structures in the CNS, the method is being used to study alterations in the <u>energy metabolism</u> of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states. | | | |

Project Description:

Previous work in this Laboratory led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{18}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate kinetically between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [^{14}C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the cerebral tissues and the hexokinase kinetic constants (V_{max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six individual ones. Further mathematical analyses revealed the way to

design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.483, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey and the cat; the values have been found to be 0.344 and 0.41, respectively. In collaboration with T. Duffy of the Department of Neurology, Cornell University, the lumped constant has been measured in the dog and found to be 0.56.

All the theoretical and technical problems were solved, and the method has now been completely operative for the last five years. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [^{14}C]deoxyglucose is injected, and arterial plasma concentrations of [^{14}C]deoxyglucose and glucose are measured from the time of injection until 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [^{14}C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped constant, local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer.

The regional localization obtained with the [^{14}C]deoxyglucose method is achieved by the use of quantitative autoradiography. The autoradiographs provide pictorial representations of the relative, not the actual, rates of glucose utilization in all structures of the brain. They are ordinarily subjected to manual densitometric analysis from which local ^{14}C concentrations are derived and used in the operational equation to compute the actual rates of local glucose utilization. We have recently developed a computerized image-processing system to analyze and transform the autoradiographs into color-coded pictorial maps of the actual rates of glucose utilization throughout the entire CNS. The autoradiographs are scanned automatically by a computer-controlled scanning microdensitometer which measures the optical density of each spot, 25-100 μm , in the autoradiograph. These optical densities are stored in a computer, converted to tissue ^{14}C concentrations on the basis of the optical densities of calibrated ^{14}C plastic standards, and then converted by the computer to actual local rates of glucose utilization by solution of the operational equation. Colors are assigned to narrow ranges of the rates of glucose utilization, and the autoradiographs are then displayed on a color monitor in color along with a calibrated color scale for identifying the rate of glucose utilization in each spot of the autoradiograph from its color. These pictures are, therefore, complete color-coded maps of the actual rates of local glucose utilization precisely localized in each

25-100 μm region of the CNS. Work is in progress to develop computerized techniques to reconstruct color maps of the entire brain three-dimensionally from the digitized autoradiographs. This would make it possible to enter into the computer all the data for the entire brain, sectioned in one plane, for example, serial coronal sections. The computer could then rotate the brain and section it from any direction, thus providing horizontal, or parasagittal sections as well. This would facilitate the identification of areas of altered cerebral metabolism in their three dimensions. The computer programming is complex, but Mr. Bruce McFarlin and Mr. Charles Goochee have made significant progress. They have developed the key elements of an algorithm to rotate images of sections, one at a time, and line them up with preceding sections. This work has been temporarily interrupted by Mr. McFarlin's recent departure.

The deoxyglucose method was originally developed for use with ^{14}C quantitative autoradiography. ^{14}C was chosen because of the availability of X-ray film sensitive to its β -radiations. The resolution of the method with ^{14}C is 50-100 μm . Because ^3H has β -radiation of considerably less energy, it is possible to achieve finer resolution, e.g., 10 μm , with [^3H]deoxyglucose, provided ^3H -sensitive film were available. X-ray film (LKB [^3H]Ultrafilm) sensitive to ^3H has now become available, and Dr. Francesco Orzi has carried out the necessary experiments to adapt the deoxyglucose method for use with ^3H . He has calibrated a set of [^3H]methyl methacrylate standards to quantify the autoradiography, and he is now in the process of applying the method to a series of normal rats to compare the results with those obtained with the [^{14}C]deoxyglucose. The autoradiographs with [^3H]deoxyglucose show much finer resolution. Structures, such as layers in the hippocampus, now show up where they were never seen before. In fact, even single stellate-shaped cells, presumably low motor neurons, have been seen in autoradiographs in the ventral horn of the cervical cord of the cat. These studies represent a significant advance in the improvement of the resolution of the [^{14}C]deoxyglucose method.

The deoxyglucose method was originally developed for use in animals in relatively normal physiological states. It was anticipated that in pathological situations, the lumped constant and rate constants determined in normal animals would not be applicable in pathological states. Experience with the method has confirmed that in severe hypoglycemia and in hyperglycemia these constants do indeed change. In the preceding year Dr. Sumio Suda determined the lumped constant in hypoglycemia, and Dr. Franz Schuier and Dr. Francesco Orzi determined the lumped constant at several different levels of hyperglycemia. During this last year Dr. Orzi and Dr. Diana Dow-Edwards have been determining the rate constants at various blood glucose levels extending to severe hyperglycemia. The studies are almost completed. The range of arterial plasma glucose concentrations covered is from 40 to 600 mg%. When these studies are completed, not only will it be possible to study the effects of extreme changes in blood glucose level on local cerebral glucose utilization, but also to apply the method to pharmacological and physiological states that markedly alter the blood glucose level, e.g., epinephrine infusions, stress, diabetic acidosis and coma, etc.

Previous studies in this Laboratory demonstrated that dopamine agonists, such as d-amphetamine or apomorphine, activated glucose utilization in all the components of the dopaminergic pathways of the extrapyramidal motor system. These effects were blocked by dopamine antagonists, such as haloperidol, which alone produced opposite effects. A surprising finding was the absence of any effects

on the dopaminergic mesolimbic system, which has been hypothesized to be over-active in amphetamine psychosis and, perhaps, also in schizophrenia. The previous studies were carried out acutely following single intravenous doses of the drugs. Amphetamine-psychosis characteristically occurs, however, after chronic use of amphetamine. Dr. Orzi and Dr. Dow-Edwards have, therefore, repeated the previous studies with d-amphetamine, but with various modes of administration of the drug. They have confirmed that acute doses of d-amphetamine do not affect the mesolimbic system, but following continuous administration by osmotic pumps, glucose utilization is markedly activated in regions of the nucleus accumbens. These results lend support to the hypothesis that chronic amphetamine administration results in increased activity in the nucleus accumbens which may underlie the development of amphetamine psychosis.

In previous years Dr. Carolyn B. Smith applied the deoxyglucose method to the problem of normal aging in rats. She found selective decreases in local cerebral glucose utilization with age with the most profound effects in all the components of the primary auditory and visual pathways. These were effects similar to those seen following sensory deprivation of these systems. These results raised the question of whether or not some of the central nervous consequences of normal aging might not be due to sensory deprivation due to sense-organ degenerative changes inasmuch as there is known to be some retinal and inner ear degenerative changes with age. Decreases in glucose utilization were also seen in the basal ganglia. These are structures which are part of the nigrostriatal dopamine system, and glucose utilization in these structures is normally activated by dopamine-agonists and depressed by dopamine antagonists. In order to determine whether the decreases with aging were due to loss of functional dopamine receptors, she initiated studies of the effects of normal aging on the metabolic responsiveness of these structures to the administration of the specific dopamine agonist, apomorphine. She has continued these studies during the past year with the assistance of Dr. Malin Ingvar and Dr. Philippe Maeder. The studies are not yet complete, but the results thus far indicate that there is a loss of responsiveness with age; in the oldest age group, 24 months and older, there is essentially no metabolic response to apomorphine. These results suggest that a loss of functional dopamine receptors in the nigrostriatal system occurs with age, a change that may help to explain senile parkinsonism.

Dr. Diana Dow-Edwards is completing a study on the effects of thyroid imbalance on local cerebral glucose utilization. Hyperthyroidism and hypothyroidism appear to have few, if any, effects on local cerebral glucose utilization in adult rats, findings that might have been expected from previous results obtained in man with methods that measured average oxygen consumption of the brain as a whole. In animals made cretinous by radiothyroidectomy at birth but allowed to reach adult age, there is a diffuse decrease in cerebral glucose utilization with some systems, particularly the auditory and visual systems, most prominently affected. Cerebral cortex in general was greatly affected, probably because of the retarded development of the neuropil which has been demonstrated by anatomical techniques.

Drs. Massako Kadekaro and Paul Gross, in collaboration with Dr. Juan Saavedra of the Laboratory of Clinical Science, have been applying the deoxyglucose method to studies of the Brattleboro rat. This is a genetic strain of the Long-Evans rat which suffers from a defect in vasopressin synthesis and exhibits a characteristic diabetes insipidus. Despite the failure in vasopressin synthesis, the

neurohypophysis shows marked increases in glucose utilization. It is as though the gland works harder because it cannot release vasopressin. Parenteral vasopressin administration in doses adequate to control the diabetes insipidus, does not reverse this metabolic activation. Other components of the hypothalamico-hypophysial tract, e.g., supraoptic and paraventricular nuclei, are not affected. The only other structure which shows an increased glucose utilization is the subfornical organ. This structure is known to mediate drinking in response to high plasma levels of angiotensin II, and the Brattleboro rat exhibits elevated drinking behavior and has high plasma concentrations of angiotensin II. The mechanism of increased glucose utilization in the neurohypophysis is under study.

Drs. Linda Porrino and Hiroki Namba have been using the deoxyglucose method to identify neuronal circuits upon which the female sex hormones, estrogen and progesterone, act to regulate sexual behavior in the female rat. In ovariectomized rats estrogen alone stimulates glucose utilization in the mid and posterior portions of the hypothalamus. Progesterone alone has no effects but when administered following pretreatment with estrogen depresses activity in the anterior-preoptic hypothalamic area. These results demonstrate an anatomical separation of the effects of female gonadal steroids in the hypothalamus and may reflect the effects of these hormones on female sexual behavior.

Dr. Charles Kennedy and Dr. Masanori Ito, in collaboration with many investigators in the Sleep Unit and Laboratory of Neuropsychology of the NIMH, have completed their studies of the relationship between slow wave (non-REM) sleep and local cerebral glucose utilization in monkey. The results demonstrate that during slow-wave sleep there is a generalized, diffuse, relatively uniform depression of cerebral glucose utilization throughout the central nervous system. There was no increase in glucose utilization in any structure, not even those alleged to be hypnogenic centers which when activated turn off activity in other regions of the CNS. These results offer no support to theories of hypnogenic centers of sleep, at least in slow-wave sleep, and are more consistent with a chemical theory in which some sleep-promoting substance is acting on neural tissue to depress activity.

The deoxyglucose method has been adapted for human use by substitution of the positron-emitting [^{18}F]fluorodeoxyglucose and positron-emission tomography for [^{14}C]deoxyglucose and autoradiography, respectively. The Nuclear Medicine Branch of the NIH has a facility available to apply this method. Members of our Laboratory are collaborating with Dr. DiChiro of the NINCDS in studies of gliomas. The results thus far show that the method is capable of detecting and localizing the sites of gliomas, in some cases in which CAT scanning failed, and can also provide an estimate of the grade of malignancy. There appears to be a direct relationship between the grade of malignancy and the rate of glucose utilization. Grade I gliomas utilize glucose at a rate similar to that of normal white matter; Grade IV tumors have an enormously elevated rate of glucose utilization. The Laboratory is also collaborating with Dr. Stanley Rapaport of the National Institute of Aging in studies of normal aging and with Dr. Monte Buchsbaum in studies of schizophrenia; the results of these studies are still insufficient for definitive conclusions.

Significance to Biomedical Research and Program of Institute.

The deoxyglucose method has made it possible for the first time to measure the rates of glucose utilization simultaneously in all functional and structural components of the central nervous system of conscious, behaving animals and now also in man. Because the method was developed in our Laboratory, it has been our responsibility to survey its applicability to the various types of conditions in which it might be applicable. The program has, therefore, been somewhat heterogeneous covering a wide range of physiological, pharmacological, pathological, and altered behavioral states. The method and its wide-ranging usefulness has now been more or less established, and it is used extensively throughout the world in neuroanatomical, neurophysiological, neuropharmacological, psychiatric, neurological, and neurosurgical research, and its wide acceptance is directly related to the results of studies in this project.

Future Course of Project.

The applications of the deoxyglucose method to studies of sexual behavior and the influence of sex steroids on local cerebral glucose utilization will be continued and extended to the effects of these hormones when administered in the critical period for determination of future sexual behavior just after birth. The studies on the effects of thyroid dysfunction on local cerebral glucose utilization will be completed, and no further studies are presently contemplated. The ³H modification of the deoxyglucose method will be completed and instituted as a routine procedure in those studies in which higher resolution is desirable. The studies of aging and the responsiveness of dopamine-receptive structures to dopamine-agonists will also be completed, but studies of aging, particularly the effects of sensory isolation, will be studied further. The studies of sleep will be continued with particular efforts to measure local cerebral glucose utilization during REM sleep. The studies on glucose utilization in the hypothalamico-hypophysial system of the Brattleboro rat will also be continued with the goal of defining the mechanism of the metabolic activation in the neurohypophysis.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00887-05 LCM |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Extended Visual System of the Macaque Monkey | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | C. Kennedy M. Mishkin | Guest Worker Research Psychologist LCM NIMH LN NIMH |
| OTHER: | M. Ito K. Macko L. Sokoloff | Visiting Associate Staff Fellow Chief, Lab. of Cerebral Metab. LCM NIMH LN NIMH LCM NIMH |
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| SECTION Section on Developmental Neurochemistry | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland | | |
| TOTAL MANYEARS: 2.0 | PROFESSIONAL: 1.25 | OTHER: 0.75 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The <u>deoxyglucose</u> method is being applied to the <u>monkey</u> to advance knowledge regarding the parts of the brain which are involved in the <u>processing of visual information</u> . By measuring rates of local cerebral glucose utilization in animals during their performance of tasks involving different types of visual stimuli we anticipate learning which parts of brain are involved in such functions as <u>discrimination</u> , <u>memory</u> and <u>motivation</u> . Also by studying animals at various ages, information will be obtained regarding the <u>maturation</u> of the visual processing system. | | |

Project Description:

The goal of the collaborative effort initiated in 1978 was to map regions of monkey brain which were responsive to visual stimulation. The deoxyglucose method has been shown to be sensitive to even small differences in functional activity, and it was hoped that it might be possible to shed light on such complex aspects of visual function as discrimination, memory, or even the mechanism by which the brain assigns a value judgement on the character of visual stimulation and then initiates a response to it. The procedure followed is to prepare animals so that one hemisphere is completely deprived of visual input. One optic tract is sectioned as is the corpus callosum and forebrain commissures. Because the intact brain functions symmetrically and therefore has equal metabolic rates in all homologous structures, the finding of right-left differences in metabolic rates in the surgically prepared animals serves to identify the visually responsive regions. The experiments to date have demonstrated that these include the striate cortex and entire expanse of the circumstriate and inferior temporal cortex as far forward as the temporal pole.

The cortical areas related to vision were found to include the entire expanse of striate, prestriate, and inferior temporal cortex as far forward as the temporal pole, the posterior part of the inferior parietal lobule, and the prearcuate and inferior prefrontal cortex; subcortically, in addition to the dorsal lateral geniculate nucleus and superficial layers of the superior colliculus, the structures related to vision included large parts of the pulvinar, caudate, putamen, claustrum, and amygdala. These results, which are consonant with a model of visual function that postulates an occipito-temporo-prefrontal pathway for object vision and an occipito-parieto-prefrontal pathway for spatial vision, reveal the full extent of those pathways and localize their points of contact with limbic, striatal, and diencephalic structures.

A major project has been the delineation of the exact border between visual and non-visual cortex throughout this extended region. This has been facilitated by the computer-assisted image-processing system which makes possible the estimation of average values for histologically distinct cortical areas. The border separating visual from non-visual cortex has now been mapped in detail through the entire extent of striate cortex (Areas OB and OA) to the inferior convexity of the temporal lobe (Areas TEO and TE).

In other experiments the contribution of the commissural systems to these visually responsive cortical areas was determined. The commissural systems are those which transmit visual information from cortex across the mid-line to contralateral cortex. This was done by comparing average rates of glucose utilization in cortex in monkeys which had the optic tract alone sectioned with those which had had optic tract section plus commissural section. The results indicated that the commissural contribution is very largely due to a region designated TE in the anterior portion of the inferior temporal lobe.

In the experiments cited above many animals had been trained to respond to a specific visual stimulus with unimanual key-pressing to obtain a water reward. Thus the same experiments which were used to map the extended visual system also provided information on the metabolic responses to motor activity. While the motor pathways of the brain have been identified by other techniques, and thus are known, these experiments served to delineate the specific subdivisions of

many structures which selectively are activated in the unimanual key-pressing. They provided new information on somatotopic localization of arm-hand movements. This was particularly well-defined in the study of cerebellar cortex. A large part of Crus II of the ipsilateral cerebellar cortex was shown to be selectively responsive in the animals' performance of the task. Also participating, but with a lesser percent change, was the lateral portion of the vermis in lobules III-VI. Localized increments in the rate of glucose utilization were also noted in VL and VPL of the thalamus, part of the globus pallidus and discrete zones of cerebral cortex (S, S_{II}, M) and a part of the supplementary motor area. A noteworthy feature of this mapping study of motor activity is that a much greater metabolic increment was found in structure concerned with sensory monitoring of motor activity than in those related to the motor activity itself.

Significance to Biomedical Research and to Program of the Institute.

This project represents a collaborative effort between the Laboratory of Cerebral Metabolism and the Laboratory of Neuropsychology in which the specialized expertise of each Laboratory is brought to bear on the use of the deoxyglucose method to study higher nervous functions, in this case the higher level processing of visual information beyond the primary visual system. The advantage of this approach is the ability to examine all local regions of the brain simultaneously in unanesthetized animals. It is hoped that these studies will help to elucidate the regions of the brain involved in integrating sensory inputs and eliciting appropriate affective responses.

Proposed Course.

The analysis of data obtained in experiments already carried out will be continued. Yet to be undertaken are experiments with different types of visual stimulation and with visual stimulation associated with tasks requiring learning and memory.

The Laboratory of Neuropsychology is reporting on this project with Report No. Z01 MH 02033-05 LN, titled "Functional Mapping of Sensory Systems".

Publications:

Kennedy, C., Miyaoka, M., Suda, S., Macko, K., Jarvis, C., Mishkin, M., and Sokoloff, L.: Local metabolic responses in brain accompanying motor activity. *Trans. Amer. Neurol. Assoc.* 105: 13-17, 1980 (copyright, 1981).

Macko, K.A., Jarvis, C.D., Kennedy, C., Miyaoka, M., Shinohara, M., Sokoloff, L., and Mishkin, M.: Mapping the primate visual system with 2-[¹⁴C]deoxyglucose. *Science* (in press) 1982.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00889-03 LCM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) A Method for the Determination of Local Rates of Protein Synthesis in Brain | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 35%;">Carolyn Smith</td> <td style="width: 45%;">Staff Fellow</td> <td style="width: 10%;">LCM NIMH</td> </tr> <tr> <td style="vertical-align: top;">OTHER:</td> <td>L. Sokoloff</td> <td>Chief, Lab. of Cerebral Metabolism</td> <td>LCM NIMH</td> </tr> <tr> <td></td> <td>C. Kennedy</td> <td>Guest Worker</td> <td>LCM NIMH</td> </tr> <tr> <td></td> <td>C. S. Patlak</td> <td>Chief, Theoretical Statistics & Math.Br.</td> <td>B NIMH</td> </tr> <tr> <td></td> <td>K. Pettigrew</td> <td>Mathem./Stat., Theor. Stat. & Math.Br.</td> <td>B NIMH</td> </tr> <tr> <td></td> <td>F. Orzi</td> <td>Visiting Fellow</td> <td>LCM NIMH</td> </tr> <tr> <td></td> <td>J. C. Gillin</td> <td>Chief, Unit on Sleep Studies</td> <td>APB NIMH</td> </tr> <tr> <td></td> <td>W. Mendelson</td> <td>Research Psychiatrist</td> <td>APB NIMH</td> </tr> <tr> <td></td> <td>F. I. Storch</td> <td>Psychology Technician</td> <td>APB NIMH</td> </tr> <tr> <td></td> <td>R. K. Nakamura</td> <td>Senior Staff Fellow</td> <td>LPP NIMH</td> </tr> <tr> <td></td> <td>M. Mishkin</td> <td>Acting Chief, Lab. of Neuropsychology</td> <td>LN NIMH</td> </tr> <tr> <td></td> <td>M. Ingvar</td> <td>Visiting Fellow</td> <td>LCM NIMH</td> </tr> <tr> <td></td> <td>H. Namba</td> <td>Visiting Fellow</td> <td>LCM NIMH</td> </tr> <tr> <td></td> <td>D. Dow-Edwards</td> <td>Staff Fellow</td> <td>LCM NIMH</td> </tr> </table> | | | PI: | Carolyn Smith | Staff Fellow | LCM NIMH | OTHER: | L. Sokoloff | Chief, Lab. of Cerebral Metabolism | LCM NIMH | | C. Kennedy | Guest Worker | LCM NIMH | | C. S. Patlak | Chief, Theoretical Statistics & Math.Br. | B NIMH | | K. Pettigrew | Mathem./Stat., Theor. Stat. & Math.Br. | B NIMH | | F. Orzi | Visiting Fellow | LCM NIMH | | J. C. Gillin | Chief, Unit on Sleep Studies | APB NIMH | | W. Mendelson | Research Psychiatrist | APB NIMH | | F. I. Storch | Psychology Technician | APB NIMH | | R. K. Nakamura | Senior Staff Fellow | LPP NIMH | | M. Mishkin | Acting Chief, Lab. of Neuropsychology | LN NIMH | | M. Ingvar | Visiting Fellow | LCM NIMH | | H. Namba | Visiting Fellow | LCM NIMH | | D. Dow-Edwards | Staff Fellow | LCM NIMH |
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| COOPERATING UNITS (if any) B. Agranoff, University of Michigan, Ann Arbor, Michigan; R. Collins, Washington University, St. Louis Mo.; M. Phelps, UCLA, Los Angeles, Calif. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Cerebral Metabolism | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Developmental Neurochemistry | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 6.0 | PROFESSIONAL: 4.0 | OTHER: 2.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> A method is being developed for the estimation of local rates of <u>protein synthesis in brain</u> in vivo. The method is based on the use of <u>L-[1-¹⁴C]leucine</u> as a tracer for the incorporation of leucine into protein. Six kinetic models for the behavior of leucine on brain have been designed. By mathematical analysis of the <u>kinetics</u> of exchange of the amino acid between plasma and the tissue pool(s) and its incorporation into protein, equations have been derived for each model that define the rate of amino acid incorporation into protein in terms of the time course of plasma-specific activity, final tissue concentration of ¹⁴C, and experimentally determined kinetic constants. Tissue concentrations of ¹⁴C are determined by <u>quantitative autoradiography</u>. Experiments are being carried out to decide which is the best <u>model</u>. </p> <p> The method is currently being applied to studies of aging, development, cretinism, plasticity in the visual system, seizures, regeneration, and sleep. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. This method is similar to the [^{14}C]deoxyglucose method in that it is based on enzyme kinetic principles as applied to the measurement of reaction rates in vivo with labeled tracers as substrates. In order to measure the rate of the reaction, one must know the amount of labeled product formed in a given interval of time and the integrated specific activity of the precursor. In an in vivo experiment the precursor pool cannot be sampled and the specific activity determined directly. It is necessary, therefore, to design a model for the behavior of the precursor in vivo and by kinetic analysis of the model to derive a relationship between the entire history of the precursor specific activity in the plasma (which can be sampled and measured directly), the integrated specific activity of the precursor pool in the tissue, and the rate of the reaction. Six kinetic models with progressively increasing complexity to take as many of the processes and factors into account as possible have been developed, and an operational equation for each model has been derived. Studies are in progress to identify the simplest model that adequately describes the processes proceeding in vivo.

With all of the models we have chosen L-[^{14}C]leucine as the radiolabeled tracer for this method because the $^{14}\text{CO}_2$ derived from its metabolism is rapidly diluted in the pool of CO_2 and cleared from the tissue. There are, therefore, no side-reactions with radioactive products other than the labeled protein. Our current and most comprehensive model (Model VI) for the behavior of leucine in brain includes an extracellular and two intracellular compartments. The intracellular compartments are the precursor pool for protein synthesis, consisting of the activated amino acid, and the metabolic pool, the receptacle for discharged amino acid and the products of protein degradation. On the basis of the results of biochemical studies reported in the literature we propose that the amino acid is activated at the cell membrane. Therefore, only amino acid derived from the extracellular pool feeds the precursor pool. This compartmentalization would preclude mixing of the leucine derived from protein degradation with the precursor amino acid pool for protein synthesis. In vivo, however, because the extracellular space is small, this mixing might occur outside the cell.

We are currently trying to test this model. Our experiment is to determine the specific activity of brain leucyl-tRNA and plasma leucine in a rat in a steady state for both labeled and unlabeled leucine in the plasma. If the leucine is reutilized, the specific activity of the leucyl-tRNA will never reach the specific activity of the plasma leucine because it will be constantly diluted by unlabeled leucine derived from protein degradation. We have worked out a schedule for the intravenous infusion of labeled leucine in order to achieve a constant plasma level. We have also developed a method for the extraction and determination of picomolar levels of leucyl-tRNA in brain. With the use of differential centrifugation, acid precipitation, and phenol extraction, yields of tRNA of 100-200 $\mu\text{g/g}$ brain can be achieved. Our best yields of leucine following deacylation of the tRNA at pH 10 are about 20 pmoles/g brain. Consequently we have had to develop an ultrasensitive method for determination of the level of leucine derived from leucyl t-RNA. The method (adapted from a published method of Airhart et al., 1974) is based on the formation of labeled fluorescent amino acid chlorides following reaction of the amino acid extract with labeled dansyl chloride. The dansylated amino acids are separated with HPLC, and the dansyl-leucine peak is

collected and counted with double label liquid scintillation counting. With this method we can detect as little as 5 pmoles of leucine. The final results from this series of experiments will provide us with an answer to the question of reutilization of leucine derived from protein degradation and the half-life of the precursor pool. With these results we can test the validity of Model VI.

Although the choice of model to be used is still undecided, we expect that this will be defined in the near future. Therefore, we have carried out some studies on protein synthesis in brain in which with some assumptions we can obtain reasonable minimal estimates of the rates of protein synthesis. The assumptions are:

- 1) a steady state for protein and amino acid metabolism
- 2) no breakdown of labeled protein
- 3) tracer kinetics
- 4) no reutilization of leucine derived from protein degradation
- 5) complete loss of $^{14}\text{CO}_2$ from the oxidation of L-[1- ^{14}C]leucine.

For these experiments we have used Model I for a single brain pool of leucine. By mathematical analysis of the kinetics of the exchange of the amino acid between plasma and tissue and its incorporation into protein, we have derived the equation that defines the rate of leucine incorporation into protein in terms of the following measurable variables: the time course of plasma-specific activity, final tissue concentration of ^{14}C , and experimentally determined kinetic rate constants. The equation defines the procedure to be used and the variables to be measured. A pulse of [^{14}C]leucine (100 $\mu\text{Ci/kg}$ body weight) is administered intravenously, and the arterial plasma concentrations of labeled and unlabeled leucine are monitored for the duration of the experimental period. At 60 minutes the animal is killed by an intravenous injection of pentothal. The brain is removed, frozen and sectioned and local tissue concentrations of ^{14}C are determined by quantitative autoradiography.

We have determined rates of protein synthesis in a number of brain regions. We find a wide range of values from 1.5 nmoles leucine/g of tissue/min in white matter to 20 nmoles/g/min in some hypothalamic nuclei (e.g., the supraoptic nuclei). In general, brain regions that are rich in nerve cell bodies, such as the pyramidal cell layer in the hippocampus, and cranial nerve nuclei such as the dorsal motor nucleus of the vagus, have high rates of protein synthesis as compared to either white matter or regions composed largely of nerve terminals, dendrites, synapses, and axons, such as the caudate nucleus, thalamus and cortex. The value that we have obtained for cortex (5.0 nmoles leucine/g/min) compares favorably with values obtained by Dunlop et al. (1975) of 4.7 nmoles valine/g/min with a completely different method that does not yield local values.

We have embarked on several studies of the effects of specific treatments or conditions on local rates of protein synthesis. The purpose of some of these studies is to test the sensitivity of the method to detect changes in local rates of protein synthesis as well as to determine the responsiveness of protein synthesis to altered physiological states or pathological conditions. Experiments done in collaboration with Dr. R. Collins have shown that chemically-induced focal seizures produce a reduction in protein synthesis while stimulating glucose utilization. Studies of the effect of injury to the hypoglossal nerve have been carried out in collaboration with Dr. B. Agranoff. These studies have shown that cutting the hypoglossal nerve on one side will result in an increase in protein

synthesis in its nucleus. We have studied the time course of this effect. We have also examined the time course of the effect of nerve section on glucose utilization in the nucleus. Our results show that the earliest change occurs in the rate of glucose utilization within 24 hours after nerve section. The increase in protein synthesis occurs later on day 4. The magnitude of the effect on glucose utilization is larger (70-80% increase over control) than that on protein synthesis (20-30%). Both of these metabolic responses return to normal by day 35. A functional connection between the nerve and tongue is restored by day 24.

In collaboration with Dr. C. Gillin, the Unit on Sleep Studies, we are also studying the effects of slow wave sleep on local rates of protein synthesis in monkey. In the first pair of animals to be analyzed the rates of protein synthesis were generally lower in the brain of the sleeping animal as compared with the control. In three of the 88 gray matter regions examined, however, there were higher rates in the sleeping monkey; the three regions so affected were the substantia innominata, area postrema and locus coeruleus.

A study on the effects of age on local rates of protein synthesis in rats is being carried out by Dr. M. Ingvar. Three groups of rats are being studied: young adult, 4-6 months; middle-aged, 15-16 months; and aged, 24-30 months. The leucine method is also being applied to the study of the effects of hypothyroidism and cretinism on protein synthesis in rat brain by Dr. D. Dow-Edwards.

The method is also being used to study maturation and plasticity of the visual system in the newborn monkey. Prolonged monocular deprivation at birth results in a broadening of the ocular dominance columns representing the intact eye at the expense of the columns of the deprived eye. Eventually most of the striate cortex may be incorporated into a monocular visual system serving only the undeprived eye. The process underlying this reorganization is unclear. There may be an accelerated growth of terminals from the functional columns into the adjacent columns or a reduced growth and consequent retraction of the terminals of the non-functional pathway or a combination of both. The perikarya of the terminals in the striate cortex are located in well-defined layers segregated for the right and left eyes in the lateral geniculate bodies. We applied the [14 C]leucine method to the study of protein synthesis in chronic and acute monocular visual deprivation in newborn monkeys. Chronic monocular deprivation resulted in decreased rates of protein synthesis in the laminae of the lateral geniculate nuclei innervated by the deprived eye whereas in geniculate laminae innervated by the functioning eye rates of protein synthesis were normal. Acute monocular deprivation produced no differential changes in rates of protein synthesis in any of the geniculate laminae. These results suggest that the underdevelopment of the deprived columns is the result of inadequate growth and/or maintenance of axon terminals with consequent default of synaptic connections to the normally maintained terminals of the functional pathway.

We are following up these studies on the monkey visual system by looking at the recovery from monocular deprivation in the lateral geniculates and the striate cortex. In a monkey in which we opened the deprived eye and sutured the previously opened eye the [14 C]leucine autoradiographs showed a reversal in the layers of the lateral geniculates, i.e., the layers innervated by the initially opened eye had lower rates of protein synthesis than those innervated by the initially deprived eye. This effect was more pronounced in the geniculate ipsilateral to the initially opened eye. Other experiments in progress are designed to further

examine the processes involved in the plasticity demonstrated by this system. In addition, we are studying older monkeys to test whether or not this plasticity exists in the fully developed visual system.

In collaboration with Dr. M. Phelps of the Division of Nuclear Medicine, UCLA, an organization that has a functional positron-emission tomographic laboratory, we are trying to adapt the method for use in man. The synthesis of [1-¹⁴C]leucine and the purification of the L-isomer have been worked out. The rate constants for our Model V have been determined in adult monkeys. With the fit to this two compartment model it appears that in adult monkey the metabolic pool has an 11-minute half-life. This presents some difficulties for the human application because the half-life of ¹¹C is only 20 min. Studies are in progress to achieve conditions which will shorten the half-life of the metabolic pool without affecting the rate of protein synthesis.

Significance to Biomedical Research and Program of the Institute.

Protein synthesis is probably the most important biochemical process underlying the development, maturation, plasticity, maintenance, and long-term regulation of the nature and degree of functional activity of the nervous system. The structural, functional, and metabolic properties of the tissues largely reflect the role of structural and enzymatic proteins. Peptides that are considered to be neurotransmitters are in some, and possibly all, cases derived from the cleavage of large parent protein molecules. Many hormones within and outside the nervous system are proteins. It is, therefore, certain that changes in protein synthesis can and do alter function and that some mental and neurological dysfunctions reflect disturbances in this vital biochemical process. This research is directed at determining the rates of protein synthesis in specific regions of the central nervous system with an ultimate resolution down to the cellular level. This provides for the first time the opportunity to study at the individual structural or anatomical level the changes in protein synthesis that may be the causes, consequences, or correlates of normal conditions, such as maturation, plasticity, differentiation, sleep, learning and memory, behavioral patterns, etc., or pathological conditions, such as hormonal disorders, aging, regeneration in response to injury, convulsive disorders, coma, etc.

Proposed Course.

We are concentrating our efforts on completing experiments on the question of dilution of the precursor pool specific activity with leucine derived from protein degradation. In the same experiments we are determining the half-life of the precursor pool. In collaboration with Drs. Patlak and Pettigrew we are determining the rate constants for our latest kinetic models. We are calibrating new [¹⁴C]methyl methacrylate standards for quantitative autoradiography with 10, 20, and 30 μ m sections of brain. These standards are in a lower range than those used in the deoxyglucose method and will be more suitable for leucine autoradiographs. In addition we are working out a reliable procedure to completely wash out free amino acid remaining in the brain following the pulse injection. This would simplify the operational equation, shorten the procedure, and require fewer assumptions for the calculation of local rates of protein synthesis, regardless of the final model adopted.

The applications of the method which are currently in progress will be continued; these include studies of CNS regeneration, slow wave sleep, aging, cretinism, and plasticity in the monkey visual system.

Publications:

Collins, R.C., Namba, H., Smith, C.B., and Sokoloff, L. Focal seizures inhibit protein synthesis. Trans. Amer. Neurol. Assoc. 105: 43-46, 1980 (copyright, 1981).

Phelps, M.E., Huang, S.C., Smith, C., Barrio, J.R., Keen, R., MacDonald, N.S., Mazziotta, J.C., Sokoloff, L. Tomographic measurement of local protein synthesis in man with 11C-L-leucine. In Heiss, W.D., Phelps, M.E. (Eds.): Positron Emission Tomography of the Brain. Berlin, Springer-Verlag, 1982, (in press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00900-26 LCM | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 through September 30, 1982</p> | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Biochemical Studies on Myelin and Myelin Basic Protein</p> | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 50%;">R. E. Martenson</td> <td style="width: 20%;">Research Chemist (Biochem.)</td> <td style="width: 20%;">LCM NIMH</td> </tr> <tr> <td>OTHERS:</td> <td>G. E. Deibler</td> <td>Chemist</td> <td>LCM NIMH</td> </tr> <tr> <td></td> <td>M. L. Pedersen</td> <td>Biologist</td> <td>LCM NIMH</td> </tr> </table> | | | PI: | R. E. Martenson | Research Chemist (Biochem.) | LCM NIMH | OTHERS: | G. E. Deibler | Chemist | LCM NIMH | | M. L. Pedersen | Biologist | LCM NIMH |
| PI: | R. E. Martenson | Research Chemist (Biochem.) | LCM NIMH | | | | | | | | | | | |
| OTHERS: | G. E. Deibler | Chemist | LCM NIMH | | | | | | | | | | | |
| | M. L. Pedersen | Biologist | LCM NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) Dr. W. J. Moore, University of Sydney Dr. H. C. Agrawal, Washington University, St. Louis, MO | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Cerebral Metabolism | | | | | | | | | | | | | | |
| SECTION Section on Myelin Chemistry | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 1.5 | OTHER: 1.0 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input checked="" type="checkbox"/> (c) NEITHER </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-start; margin-top: 5px;"> <div> <input type="checkbox"/> (a1) MINORS </div> <div> <input type="checkbox"/> (a2) INTERVIEWS </div> </div> | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Three avenues of work are being pursued: (1) <u>specific cleavages</u> of the central nervous system myelin basic protein to obtain suitable fragments for biochemical and immunological studies, (2) identification of the specific amino acids in this protein that are phosphorylated <u>in vivo</u>, and (3) theoretical studies aimed at elucidating the secondary and tertiary structures of the myelin basic protein specific to the peripheral nervous system.</p> | | | | | | | | | | | | | | |

Project Description:

We have recently isolated and purified a number of peptic fragments of the myelin basic protein of rabbit brain. In a collaborative study with Dr. Walter Moore at the University of Sydney, these fragments are being used in nuclear magnetic resonance (NMR) studies to help elucidate the detailed conformation of the protein. Some of these fragments, along with additional fragments and intact basic proteins of other species, have been used to assign the different (but closely spaced) threonine γ -CH₃ resonances in the NMR spectrum to specific Thr residues in the protein. Since fragments can be chosen that contain either no or some Thr residues in common, and since the positions of the Thr residues in the amino acid sequences of the proteins are known, comparisons of the NMR spectra make the assignment possible.

The particular geometry and environment of a residue in a protein influences the extent to which its resonance peak is shifted from its position as a free amino acid. The occurrence of several distinct threonine γ -CH₃ resonances in the NMR spectrum of the basic protein indicates that these Thr residues are physico-chemically non-equivalent. Thus, when the conformation of the protein changes from a largely unordered one in aqueous solution to a partially helical one upon binding to phospholipid micelles, it should be possible to use the Thr residues as "reporter" groups to determine which parts of the protein undergo conformational transitions.

A second avenue of work has involved examinations of the basic protein with regard to the positions of phosphoserine and phosphothreonine in the amino acid sequence. By isolating peptic and tryptic peptides of the rabbit protein and examining them for these two amino acids, we have found five phosphorylated sites: Ser-7, Ser-56, Thr-96, Ser-113 and Ser-163. Previous work by others had revealed that only Thr-96 and Ser-163 were phosphorylated in vivo. This work is particularly significant in that it adds a new dimension to earlier and current studies in other laboratories showing rapid in vivo incorporation of ³²P into the myelin basic protein. In addition, it will now be possible to compare the in vivo phosphorylation sites with sites phosphorylated in vitro with different protein kinases. The comparison should show which kinase(s) could be involved in the physiological phosphorylation of the basic protein and, moreover, give an indication of the substrate specificity of the kinase(s) with regard to the amino acid sequence around the phosphorylation site.

Additional studies have been carried out in collaboration with Dr. Harish Agrawal at Washington University. These have shown that in 15-day-old rabbits, the ³²P that is incorporated into the basic protein is present only in Components 3, 5, and 7, which have a relative specific activity of approximately 1:2:3. This finding is consistent with the generation of these less basic species by successive incorporation of 1, 2, and 3 phosphate groups into the unmodified species, Component 1. These studies indicate that it should be possible to determine the relative degree of mono-, di-, and tri-phosphorylation of the protein that occurs in vivo.

Finally, the theoretical study of the structure of the P2 myelin protein has been completed. This protein is present in peripheral nerve myelin and induces allergic neuritis in experimental animals. By the use of statistical and information theory predictive algorithms, together with the alternation of hydrophobic and hydrophilic residues along the sequence, it has been possible to predict the locations of 10 sequential β -strands a to j in the polypeptide chain. The major obstacle in predicting a tertiary structure has been the lack of definitive criteria by which to order the strands into β -sheets. Although several tertiary structures have a high degree of probability, the structure which appears most likely is a "sandwich" consisting of two antiparallel pleated sheets, one in which the strands are ordered edfgh and the other in which the strands are ordered cbaij. In this structure the disulfide bond lies in the solvent-exposed loop connecting strands i and j, while the relatively long sequence connecting strands e and f in parallel lies on the external surface of the protein. Binding of the protein to phospholipid vesicles is reported to result in some α -helix formation. In the proposed structure the helix could form in the e-f interstrand connection and propagate through most of edge strand e without causing any rearrangement of the remaining polypeptide chain.

Significance to Biomedical Research and to Program of the Institute

Knowledge gained from the studies described above are essential to an understanding of how the myelin sheath is assembled and maintained and, in addition, will provide insight into the nature of some of the pathological processes involving loss of myelin, in particular, plaque formation in multiple sclerosis.

Proposed Course

We plan to continue our studies on the chemistry of the myelin basic protein, with immediate emphasis on the effects of phosphorylation on the secondary structure of the protein as monitored by NMR spectroscopy. In addition, we wish to explore the nature of basic protein dimerization and the residues involved in the intermolecular contacts, since dimerization of the protein across the cytoplasmic surfaces of the myelin lamellae could be a mechanism for myelin compaction.

Publications:

Martenson, R. E., Law, M. J., Deibler, G. E., and Lüthy, V.: Isolation and identification of large overlapping fragments of rabbit myelin basic protein produced by limited peptic hydrolysis. J. Neurochem. 37: 1497-1508, 1981.

Martenson, R. E.: Posttranslational modifications of myelin basic protein. In Peeters, H. (Ed.): Protides of the Biological Fluids. New York, Pergamon Press, in press.

Agrawal, H. C., Martenson, R. E., and Agrawal, D.: In vivo incorporation of [³²P]-orthophosphate into myelin basic protein of developing rabbit brain. Its location in Components 3 and 5 and in a new protein tentatively identified as basic protein Component 7. J. Neurochem., in press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00901-27 LCM | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Immunologic Reactivity of Myelin Basic Protein | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: M. W. Kies</td> <td style="width: 33%;">Chief, Section on Myelin Chemistry</td> <td style="width: 33%;">LCM NIMH</td> </tr> <tr> <td>OTHER: T. Namikawa</td> <td>Visiting Fellow</td> <td>LCM NIMH</td> </tr> </table> | | | PI: M. W. Kies | Chief, Section on Myelin Chemistry | LCM NIMH | OTHER: T. Namikawa | Visiting Fellow | LCM NIMH |
| PI: M. W. Kies | Chief, Section on Myelin Chemistry | LCM NIMH | | | | | | |
| OTHER: T. Namikawa | Visiting Fellow | LCM NIMH | | | | | | |
| COOPERATING UNITS (if any) E. C. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash. | | | | | | | | |
| LAB/BRANCH Laboratory of Cerebral Metabolism | | | | | | | | |
| SECTION Section on Myelin Chemistry | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 1.5 | OTHER: 1.0 | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Transfer of EAE with BP-sensitized Lewis rat spleen and lymph node cells has been used to study <u>immune mechanisms</u> in the <u>induction and suppression of EAE</u> . Specifically, we have attempted to define the conditions under which culture with nonspecific mitogen, Concanavalin A (Con A), activates <u>BP-sensitized spleen and LN cells</u> . | | | | | | | | |

Project Description:

We previously reported that both spleen cells and lymph node cells (LNC) from myelin basic protein (BP)-sensitized Lewis rats can be activated by culture with BP. Activated cells are capable of transferring disease 100 times more efficiently than cells that have not been cultured with antigen. Cellular proliferation, measured by tritiated thymidine (^3H -TdR) uptake is also enhanced by addition of BP to cultured cells.

Spleen cells from BP-sensitized Lewis rats will also transfer EAE after culture with the T-cell mitogen, Con A. This enhancement of EAE transfer resembles but is not necessarily identical to specific enhancement by the antigen, BP. In studying the mechanism of Con A enhancement of BP-sensitized lymphoid cells, we have made the following observations:

There is a significant difference between spleen and LNC with regard to their ability to respond to Con A with enhanced transfer of EAE. Both cell populations respond to 1 $\mu\text{g}/\text{ml}$ Con A with marked cell proliferation but transfer of EAE with LNC is not enhanced, as it is with spleen cells.

Recent findings that supernatants from spleen cells cultured with Con A (SpC/Con A Sup) contain several immunologically active factors suggested to us that Con A-induced transfer with BP/CFA spleen cells is mediated by a soluble factor. Since Con A does not enhance transfer EAE with LNC, the latter cells either cannot produce the factor or cannot respond to it. We believe that they do not produce the factor, because spleen cells, either sensitized or naive, do produce soluble factor(s?) in response to Con A and LNC do respond to the soluble factor(s) produced by spleen cells.

In the experiments described above BP/CFA LNC were precultured in RPMI/FCS for 48 hours prior to addition of SpC/Con A Sup. Later experiments showed that freshly harvested LNC do not respond to SpC/Con A Sup as effectively as pre-cultured LNC, perhaps because preculture facilitates the expression of a receptor for the soluble factor on LNC. Finally, precultured BP/CFA LNC respond to some degree to 1 $\mu\text{g}/\text{ml}$ Con A with enhanced transfer. This means that preculture in RPMI/FCS not only provides some advantage to "factor-responding" LNC but also to "factor-producing" LNC.

Since BP/CFA spleen cells are enhanced by Con A, we assumed they would also be responsive to SpC/Con A Sup. Much to our surprise we found that they did not respond to SpC/Con A Sup unless they were pulsed (4 hours) with Con A before the supernatant was added. Spleen cells pulsed with Con A alone do not transfer EAE, even though these cells are able to proliferate as well as spleen cells incubated 48 hours with Con A.

One of the best characterized of the soluble factors induced by Con A stimulation of spleen cells is IL-2 (T-cell growth factor). IL-2 has been partially characterized and is known to be extremely stable. Although stimulation of IL-2 production appears to provide an adequate explanation for enhanced transfer in the case of spleen cells incubated with Con A, we believe that our results

suggest a much more complex mechanism: (1) IL-2 is stable to freezing, storage at 4°C, lyophilization, etc.; in contrast, the ability of SpC/Con A Sup to enhance transfer of EAE is very labile. We have never observed enhanced transfer with stored or frozen preparations. (2) Four hours exposure of spleen cells to Con A induces IL-2 production (and proliferation) but does not enhance transfer of EAE. (3) LNC produce IL-2 (and proliferate) when stimulated directly by Con A but require a pre-incubation period with RPMI/FCS before responding to Con A with enhanced transfer. More work is required to establish the exact nature of the "defect" in the transfer mechanism which is overcome by the addition of Con A to spleen cells or SpC/Con A Sup to LNC.

Significance to Biomedical Research and to Program of the Institute

This work represents a big step forward in our understanding of pathogenetic mechanisms which are important, not only in the experimental disease, allergic encephalomyelitis, but also in human idiopathic conditions thought to be caused by autoimmune reactions. In the latter, the mechanism of sensitization, the effect of lymphokines in the reticuloendothelial system, and the initiation of the lesion in the target organ are all completely unknown. It has been an accepted concept for many years that multiple sclerosis may be an autoimmune disease. More recently, several investigators have suggested that Alzheimer's disease may also be a condition resulting from an adverse autoimmune reaction.

Proposed Course

Studies are continuing either to identify the soluble activity with IL-2, or to establish its unique character. With the enhanced transfer technique we also hope to induce true demyelination in rats (as opposed to the more common inflammatory lesion). Preliminary data indicate that demyelination does occur after repeated transfer of BP-sensitized cells into the same recipient.

Publications:

Richert, J. R., Namikawa, T., Kies, M. W., and Alvord, E. C., Jr.: Failure of serum from recovered rats to prevent enhanced adoptive transfer of experimental allergic encephalomyelitis. J. Neurol. Sci. 53: 225-232, 1982.

Namikawa, T., Richert, J. R., Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Transfer of allergic encephalomyelitis with spleen cells from donors sensitized with myelin basic protein in incomplete Freund's adjuvant. J. Immunol. 128: 932-934, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 00902-17 LCM |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE) | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div> PI: B. F. Driscoll Guest Worker OTHER: M. W. Kies Chief, Section on Myelin Chemistry </div> <div style="text-align: right;"> LCM NIMH LCM NIMH </div> </div> | | |
| COOPERATING UNITS (if any) National Multiple Sclerosis Society E. C. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash. | | |
| LAB/BRANCH Laboratory of Cerebral Metabolism | | |
| SECTION Section on Myelin Chemistry | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 1.0 | OTHER: 1.5 |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input checked="" type="checkbox"/> (c) NEITHER </div> </div> | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p style="margin-left: 40px;"> In attempting to dissect the various cellular events involved in the <u>pathogenesis of EAE</u> we have used the phenomenon of <u>enhanced transfer</u> to study: 1) The Ia type of cells involved in transfer (whether Ia- or Ia+); 2) the occurrence of suppressor cells in cell populations obtained from protected or suppressed animals; and 3) the induction of <u>chronic EAE</u> in recipients of sub-optimal numbers of BP-sensitized cells. </p> | | |

Project Description:

EAE is induced in susceptible animals by injection of purified myelin basic protein (BP) in complete Freund's adjuvant (CFA). The subsequent immune response leading to the development of disease has been shown to be a T-cell mediated delayed type hypersensitivity (DTH) reaction. DTH in EAE can be monitored by several different techniques:

1. The development of a positive skin reaction to antigen (i.e., animals sensitized with BP/CFA have a tuberculin-type skin reaction to BP just before onset of illness).
2. Proliferation of lymphocytes in the presence of antigen in vitro.
3. Induction of EAE in naive animals with cells from BP/CFA-sensitized donor animals.

Until recently, the latter method possessed considerable technical difficulty because of the large number of cells required. Dr. Driscoll discovered about 3 years ago that very low numbers of peritoneal exudate cells (PEC) from BP/CFA sensitized guinea pigs can transfer severe EAE after culture with antigen (BP). The advantage resulting from culture with BP is at least 100-fold. Lymph node cells (LNC), in contrast to PEC, require a second non-specific stimulus for enhancement of transfer. This can be provided by the presence in culture of allogenic (Strain 2) PEC which induce a strong proliferative response in the Strain 13 LNC, known as a mixed lymphocyte reaction (MLR).

Immune reactions, like many other biological phenomena, depend upon cellular recognition and communication. In the development of an immune reaction, antigen is taken up by an antigen presenting cell (APC) and presented to a T cell. Efficient presentation of antigen requires that the T cell and the APC share certain cell surface markers (for "recognition"). These markers are glycoproteins encoded by the immune response genes which are located in the I region of the major histocompatibility gene complex (MHC). They are known as Ia (I region associated) antigens. All of the reactions involving sensitized T cells and the various stimulatory or inhibitory populations of cells with which they interact require that the interacting cells share at least some Ia antigens.

Of particular significance to our study of EAE is the observation that DTH can be transferred between various inbred strains of mice only if the donor and recipient share at least some genes in the I region of the MHC. Thus the I region and the Ia cell surface markers play a central role in the control of EAE.

There are available at NIH two strains of guinea pigs (Strain 13 and Strain 2) which share identical MHC genes except for the I region. While the two strains express some shared Ia antigens, the Strain 13 cells have Ia-1,3,7 antigens not found on Strain 2 cells and Strain 2 cells have Ia-2,4 antigens not found on Strain 13 cells. Taking advantage of these known differences, we were able to induce anti-Ia-1,3,7 antiserum (in Strain 2 animals) and anti-Ia-2,4 antiserum (in Strain 13 animals) by immunization of either strain with lymphoid cells of the other strain. These antisera have been used to ascertain the role of Ia+ cells in EAE, and in particular the role of such cells in enhanced transfer.

We reported last year that Ia antigens are already expressed on BP-sensitized T cells present in PEC in vivo (prior to culture) but that BP-sensitized LNC express Ia only after stimulation in culture. In continuing this study of the role of Ia antigens in the enhanced transfer phenomenon we have found that BP has to be presented to LNC in culture by Ia+ Strain 13 APC. The naive Strain 2 PEC were unable to present antigen to Strain 13 LNC; their function in culture was merely to induce an MLR (which presumably caused production of soluble factors essential for proliferation or differentiation of the BP-specific effector cells, thus enhancing their ability to transfer EAE).

A second question under investigation is the role of suppressor cells in the pathogenesis of EAE. In a search for such cells, Dr. Driscoll discovered that transfer of a suboptimal number of EAE effector cells (10-20% of the cells required for good disease induction in recipients) prevented subsequent active induction of EAE when the recipients were challenged with BP/CFA. These cells are not suppressor cells--the same cells when transferred in larger numbers induce disease in the recipients--but a suboptimal transfer interacts with the recipients' own cells in some manner so that active disease induction is ineffective. One hypothesis we tested was that suppressor cells were being produced in the recipient. However, we were unable to demonstrate such cells in either spleen or LNC populations. This was done by transferring cells directly from the first (suppressed) recipient into a second naive recipient and challenging the second recipient. There was no interference with EAE induction in the second recipient. In fact, the PEC of the primary suppressed recipients could induce EAE in secondary recipients after the PEC had been cultured with BP.

When whole guinea pig cord in CFA was used to challenge the "suppressed" recipients, protection was only 50% effective and the protected survivors developed a chronic type of EAE. Although this form of EAE has been promoted in recent years as a "better model for MS" than acute EAE, we had continued to study acute EAE because it is a better defined system for dissecting immune mechanisms than the chronic disease. Until we discovered the role of suboptimal transfer in the induction of chronic EAE it was a rather unpredictable phenomenon which occurred only in juvenile (<250 gm) guinea pigs. Our clinical observations on these partially suppressed animals have convinced us we are studying the same phenomenon as others have described in juvenile guinea pigs; histologic studies by Dr. Alvord support this conclusion. The lesions induced in the chronic animals resemble the large demyelinated plaques which are the hallmark of multiple sclerosis. Further, the destruction is clearly limited to myelin and does not primarily involve the axons. The latter remain intact although they are swollen and have obviously reacted to loss of the myelin sheath.

Significance to Biomedical Research and to Program of the Institute

This system permits for the first time an examination of the steps involved in the induction of chronic EAE: BP sensitized cells are clearly involved--yet, it is obvious that the initiation and growth of the large demyelinating plaque is dependent on some other phenomenon related to sensitization with whole tissue. We believe that the induction of chronic EAE by suboptimal transfer of cells is one of the most significant observations made by our group, second only to our discovery of myelin basic protein as the encephalitogen. Because it offers a

means of studying the mechanism of plaque formation, it offers also a means of interrupting the process. This should have great importance in developing a protocol for treatment of multiple sclerosis patients.

Proposed Course

The mechanism of chronic EAE is not completely clear at this point, but the study should be continued because of its value in helping us to understand the causes of human demyelination.

Publications:

Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Suppression of acute experimental allergic encephalomyelitis in guinea pigs by prior transfer of suboptimal numbers of EAE-effector cells: induction of chronic EAE in whole tissue-sensitized guinea pigs. J. Immunol. 128: 635-638, 1982.

Kies, M. W. and Driscoll, B. F.: Immunologic reactivity of myelin basic protein in inbred guinea pigs. In Peeters, H. (Ed.): Protides of the Biological Fluids. New York, Pergamon Press, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00903-05 LCM | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 through September 30, 1982</p> | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Preparation and Identification of Peptides Derived From Myelin Basic Protein</p> | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: G. E. Deibler</td> <td style="width: 33%;">Chemist</td> <td style="width: 33%;">LCM NIMH</td> </tr> <tr> <td>OTHER: M. W. Kies</td> <td>Chief, Section on Myelin Chemistry</td> <td>LCM NIMH</td> </tr> </table> | | | PI: G. E. Deibler | Chemist | LCM NIMH | OTHER: M. W. Kies | Chief, Section on Myelin Chemistry | LCM NIMH |
| PI: G. E. Deibler | Chemist | LCM NIMH | | | | | | |
| OTHER: M. W. Kies | Chief, Section on Myelin Chemistry | LCM NIMH | | | | | | |
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| COOPERATING UNITS (if any) | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Laboratory of Cerebral Metabolism</p> | | | | | | | | |
| SECTION <p style="text-align: center;">Section on Myelin Chemistry</p> | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | |
| TOTAL MANYEARS: <p style="text-align: center;">2.5</p> | PROFESSIONAL: <p style="text-align: center;">1.0</p> | OTHER: <p style="text-align: center;">1.5</p> | | | | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Myelin basic protein (BP) presents a unique opportunity for a detailed study of the relationship between <u>structure</u> and <u>biological activity</u>--BPs from several species have been completely sequenced and there is an impressive fund of information on its encephalitogenic activity in several species, its ability to induce and bind antibody and its ability to bind other biochemical compounds or structures, specifically membrane constituents.</p> <p>In order to study the <u>relationship between structure and activity</u>, we have prepared several well-characterized fragments of the protein by controlled proteolytic digestion. Our purpose is to test the ability of these peptides to replicate the biological activity of the protein. By using fragments from different regions of the protein one can "map" <u>active sites</u> and define the <u>minimal structural requirements</u> for activity.</p> | | | | | | | | |

Project Description:

Basic protein can be selectively cleaved by pepsin to yield two peptides, only one of which is encephalitogenic (Res 89-169). Recent *in vitro* studies suggested that the non-encephalitogenic half of the molecule (Res 1-88) was contaminated with a very minute amount of material derived from the encephalitogenic portion of the molecule.

Since purification by ion-exchange chromatography and gel filtration did not remove the contaminant responsible for the unexpected biological activity in peptic peptide 1-88, a method for purification by high pressure liquid chromatography (HPLC) was developed. Peptides of myelin basic protein are very basic and bind to the free silanol of the C₁₈ column which makes fractionation by HPLC difficult. To alleviate the binding, triethylamine was added to the buffer, trifluoroacetic acid. Gradient elution with acetonitrile yielded incomplete separation of peptides (89-169) and (1-88). Better analytical separation was obtained by using a combination of isocratic conditions followed by gradient elution with acetonitrile.

Twelve 200 µg aliquots of peptide (1-88) were subjected to HPLC purification and 6 different fractions were collected automatically and pooled. Each pooled fraction was checked for the *in vitro* cell proliferative response. Fractions 4-6 showed no proliferative response.

The HPLC purification of peptide (1-88) was also monitored by a very sensitive antibody blotting technique. Electrophoresis was carried out in 15% slab polyacrylamide gels and the peptides transferred to nitrocellulose membranes by electro-blotting. The transferred peptides were treated with rabbit antibody to peptide (89-169). Subsequently, peptides which reacted with specific antibody were detected by incubation with peroxidase-labelled goat anti-rabbit IgG [F(ab)₂]. Bands which bound the peroxidase-labelled antibody were stained with 3,3'-diaminobenzidine and hydrogen peroxide. Only fraction 6 obtained from the HPLC purification of peptide 1-88 contained peptides which reacted with anti-(89-169). It was estimated that the concentration of this impurity in the original preparation was approximately 0.2%.

Further Information on the Sequence of Guinea Pig BP

Although guinea pig myelin basic protein (BP) has been used in many immunologic studies, this protein has never been completely sequenced. Comparison of tryptic maps of bovine peptic peptide (1-88) and guinea pig peptic peptide (1-88) confirmed the sequence differences previously reported by Martenson and Deibler and others. Amino acid compositions and tryptic maps of guinea pig (89-169) and bovine (89-169) have also been compared. The comparison of the tryptic peptides showed that Ser (131) as well as the Ala-His sequence (136-137) are missing from the guinea pig molecule. Leu (140) is replaced by a Phe. The single His present in guinea pig peptide (89-169) is located in peptide (142-151) which also contains an extra Ala. Confirmation of these sequence changes was obtained by tryptic digestion of guinea pig V8 peptides (89-118) and (119-169). Amino acid compositions of the tryptic peptides of peptide (89-118) were identical with those

from the same region of bovine BP. The composition of the tryptic peptides of peptide (119-169) showed the same sequence changes observed in the tryptic peptides of guinea pig (89-169). In order to locate the exact positions of the sequence changes in guinea pig peptide (89-169), tryptic peptides of guinea pig V8 peptide (128-169) were eluted from several peptide maps, and their amino acid compositions determined. The 3 tryptic peptides which contained the sequence changes were given to Dr. Henry Krutsch of NIAID for sequence analysis with dipeptidyl peptidase. His analysis confirmed the location of the Ser deletion (131), the Ala-His deletion (136-137) and gave the position of the extra Ala between Gly (142) and His (143).

Significance to Biomedical Research and to Program of the Institute

The analytical methods which have been developed in our group are extremely important tools, specifically adapted for studying myelin, its synthesis, breakdown and repair. In addition to HPLC, gel electrophoresis, tryptic mapping and amino acid analysis, we have adapted antibody-blotting techniques, in vitro cell reactions, and ELISA technique for specific antibody detection.

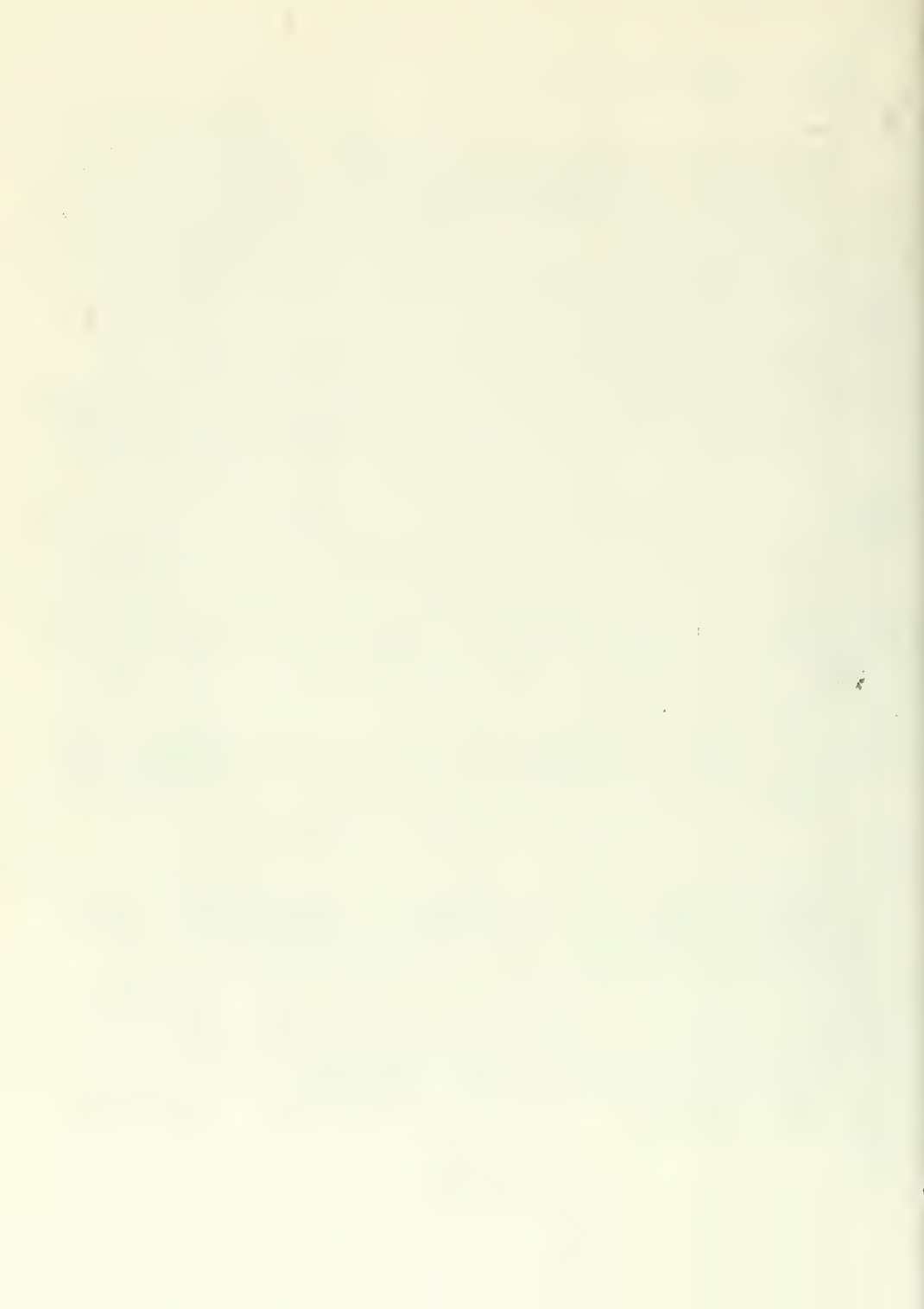
These analytical tools permit us to study a problem in depth--e.g., only by very careful dissection were we able to prove that the in vitro cellular response to peptide 1-88 of BP was caused by a contaminant, not by the peptide itself. This was important in Dr. Driscoll's interpretation of the results which were obtained in his study on pathogenesis of EAE. Eventually he hopes to identify the subset(s?) of T-lymphocytes which are involved in the induction of EAE. This knowledge will be of use to scientists investigating the role of cellular sensitivity in multiple sclerosis and other autoimmune diseases.

Proposed Course

The two problems described in this report are essentially solved, but the project itself is an open-ended one--investigation of the relationship between structure and biological activity of an important highly reactive basic protein in myelin. Projects currently under exploration involve collaboration with other laboratories as well as those originating in our own Section.

Publications:

Deibler, G. E., Nomura, K., and Kies, M. W.: Limited digestion of guinea pig myelin basic protein and its carboxy-terminal fragment (residues 89-169) with Staphylococcus aureus V8 protease. J. Neurochem., in press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00931-09 LGCB | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Studies on the Characteristics and Regulation of <u>S</u>-Adenosylhomocysteine Hydrolyase | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: G. L. Cantoni</td> <td style="width: 33%;">Chief, Lab. Gen. Comp. Biochem.</td> <td style="width: 33%;">LGCB NIMH</td> </tr> <tr> <td>R. R. Aksamit</td> <td>Senior Staff Fellow</td> <td>LGCB NIMH</td> </tr> <tr> <td>P. S. Backlund, Jr.</td> <td>Staff Fellow</td> <td>LGCB NIMH</td> </tr> <tr> <td>I.-K. Kim</td> <td>Visiting Fellow</td> <td>LGCB NIMH</td> </tr> </table> | | | PI: G. L. Cantoni | Chief, Lab. Gen. Comp. Biochem. | LGCB NIMH | R. R. Aksamit | Senior Staff Fellow | LGCB NIMH | P. S. Backlund, Jr. | Staff Fellow | LGCB NIMH | I.-K. Kim | Visiting Fellow | LGCB NIMH |
| PI: G. L. Cantoni | Chief, Lab. Gen. Comp. Biochem. | LGCB NIMH | | | | | | | | | | | | |
| R. R. Aksamit | Senior Staff Fellow | LGCB NIMH | | | | | | | | | | | | |
| P. S. Backlund, Jr. | Staff Fellow | LGCB NIMH | | | | | | | | | | | | |
| I.-K. Kim | Visiting Fellow | LGCB NIMH | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry SECTION Sections on Proteins | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2 | PROFESSIONAL: 2 | OTHER: 0 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> A large number of analogs of <u>adenosine</u> or of <u>adenosylhomocysteine</u> have been examined for their ability to function as <u>inhibitors</u> and/or <u>substrates</u> of <u>adenosylhomocysteinase</u>. The synthesis of analogs of adenosylhomocysteine by this <u>enzyme in vivo</u> may be used to form very <u>potent</u> and <u>specific inhibitors</u> of <u>trans-methylation reactions</u>. </p> <p> The two most interesting compounds studied are <u>3-deazaadenosine</u> and <u>3-deaza-aristeromycin</u>. Both are inhibitors of adenosylhomocysteinase, but only 3-deazaadenosine is a substrate for the enzyme. <u>In vivo</u>, both 3-deazaadenosine and 3-deazaaristeromycin inhibit a variety of methylation reactions, but there are differences in specificity. Both compounds exhibit <u>antiviral activity</u> against a number of <u>RNA and DNA viruses</u>. 3-Deazaadenosine, but not 3-deazaaristeromycin inhibits chemotaxis by a mouse macrophage cell line. 3-Deazaadenosine specifically inhibits synthesis of a small number of proteins, which may be due to inhibition of some reaction involved in <u>mRNA synthesis or processing</u>, such as <u>methylation of the 5' cap</u>. Differences in specificities of these compounds can be used to search for the function of specific methylation reactions in <u>chemotaxis</u>, <u>RNA synthesis</u>, and <u>other cellular processes</u>. </p> | | | | | | | | | | | | | | |

Project Description:

As is well known, S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious that the utilization of AdoMet in biological systems must be under regulatory controls, but at the present time little is known about the nature of these regulatory controls.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. For instance, the significance of the methylation of a variety of informational macromolecules, such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccharides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc., is not immediately obvious and is the subject of much debate.

It has been established that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as a methyl donor, is a competitive inhibitor of most reactions in which AdoMet participates. From the result of work in this and other laboratories, it has been proposed that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions, and plays a role in determining the hierarchy of biological methylation reactions. It can also be surmised that modulation of AdoMet/AdoHcy ratio would result in important physiological effects, which if correlated with biochemical data would help reveal the significance of specific methylation reactions.

In prokaryotes, the isolation of mutants has helped to analyze the functions of specific biochemical reactions. In eukaryotes, isolation of mutants is more difficult, so other approaches have been devised, such as using inhibitors to block specific reactions. In eukaryotes, AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteine hydrolyase (AdoHcyase), an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. We decided some years ago to take advantage of this fact and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula S-purinylnhomocysteine (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies, confirmed and extended in other laboratories, have shown that inhibitors of AdoHcyase may be divided into two groups: a) irreversible or suicidal inhibitors, that bind to the active center of the enzyme and inactivate it irreversibly, and b) competitive inhibitors that inhibit the enzyme reversibly. This second group can be further classified into two subgroups; those inhibitors which can be utilized as substrates by the enzyme and those inhibitors which are not substrates.

Several compounds have been examined which are irreversible inhibitors of AdoHcyase, and include the compounds 9- β -D-arabinofuranosyladenine (Ara-A), 3-deaza-9- β -D-arabinofuranosyladenine (3-deaza-Ara-A), and 2-chloroadenosine. Ara-A has been used by others in chemotherapy for cancer patients. 3-Deaza-Ara-A and 2-chloroadenosine might be expected to have clinical effects similar to Ara-A, since they produce similar inhibition of AdoHcyase.

Of the reversible inhibitors, two compounds have been extensively studied in this laboratory as prototype compounds of this group; 3-deazaadenosine (3-deaza-Ado) and 3-deazaaristeromycin (3-deaza-Ari). 3-Deaza-Ado is a potent competitive inhibitor of AdoHcyase with a K_i of 5-8 μ M, and as a substrate it is about equivalent to the natural substrate, adenosine with a similar affinity for the enzyme. In contrast to 3-deaza-Ado, 3-deaza-Ari is not a substrate for AdoHcyase, but it is a very potent competitive inhibitor, with a K_i of 2.0 μ M for the hamster liver enzyme.

In vivo, administration of 3-deaza-Ado to laboratory animals or cells in culture results in the accumulation of both 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) and AdoHcy. The accumulation of 3-deaza-AdoHcy can be increased by addition of homocysteine, due to AdoHcyase acting in reverse of the normal hydrolytic direction. Administration of 3-deaza-Ari brings about an increase in AdoHcy, due to the inhibition of AdoHcyase. Since 3-deaza-Ari completely inhibits AdoHcyase, the amount of AdoHcy which accumulates reflects the rate of transmethylation reactions, and not the catalytic rate of AdoHcyase.

It would be expected that the intracellular accumulation of AdoHcy and/or 3-deaza-AdoHcy, with the accompanying changes in the AdoMet/AdoHcy ratio, would result in the inhibition of a number of transmethylases. This should cause an increase in the intracellular level of AdoMet (as a consequence of its under-utilization) and in a decrease in the intracellular concentration of many methylated intermediates. We have been able to verify this prediction, demonstrating a striking decrease in the amount of many methylated compounds, including methylated phospholipids, methylated proteins, and creatine in the liver.

Studies in this and other laboratories on a large number of analogs of AdoHcy analogs have demonstrated a wide range in the sensitivity of different trans-methylases to inhibition by these compounds in vitro. Unfortunately, cellular membranes are relatively impermeable to AdoHcy and its analogs, so it has been difficult to take advantage of the specificities of these analogs in vivo. However, the ability of AdoHcyase to synthesize AdoHcy analogs in vivo, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly. Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to 3-deaza-AdoHcy being a more potent and specific inhibitor of some transmethylation reactions than AdoHcy.

We have found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-deaza-AdoHcy, brought about by treatment of the cells with 3-deaza-Ado, while chemotaxis is unaffected by accumulation of AdoHcy by treatment with 3-deaza-Ari. We have further shown that inhibition of chemotaxis is correlated with inhibition of the synthesis of specific proteins by treatment with 3-deaza-Ado, which are not inhibited by 3-deaza-Ari.

Both 3-deaza-Ado and 3-deaza-Ari inhibit various RNA and DNA viruses, however, the sensitivity of various viruses to these two drugs is different. In addition, the synthesis of cellular RNA in macrophage cells is inhibited to a greater extent with 3-deaza-Ado than with 3-deaza-Ari. The specific reaction(s) involved in inhibition of RNA synthesis has not been identified.

Comparison of the effects of 3-deaza-Ado and 3-deaza-Ari on the replication of RAW264 cells showed that, at sufficiently high concentrations, 3-deaza-Ado was cytolytic after one day and that 3-deaza-Ari was cytostatic. Micromolar homocysteine reversed the cytostasis of 3-deaza-Ari, but did not reverse the cytotoxicity of 3-deaza-Ado. By analogy to the selective inhibition of chemotaxis by 3-deaza-Ado, the cytotoxicity of 3-deaza-Ado is likely mediated by 3-deaza-AdoHcy. On the other hand, cytostasis of 3-deaza-Ari was due to a profound if not complete inhibition of AdoHcyase. Since AdoHcy is the source of homocysteine, cells incubated with 3-deaza-Ari cannot recycle methyltetrahydrofolate and regenerate tetrahydrofolate for use in *de novo* synthesis of purines and pyrimidines. In addition it would be expected that cells incubated with 3-deaza-Ari would contain less cystathionine, an amino acid without a known function that is found in high concentration in the brain. These findings could have clinical significance in situations where AdoHcyase is inhibited such as the administration of Ara-A and patients with adenosine deaminase deficiency.

Significance of Biological Research to the Program of the Institute:

Studies of the AdoHcyase and its inhibitors are important to understanding the regulation and function of biochemical transmethylation, and have possible clinical applications in the development of specific inhibitors for certain methylation reactions. Since AdoMet dependent methylation reactions are involved in the synthesis of so many compounds, including DNA, RNA, proteins, lipids, and neurotransmitters, the regulation of these reactions can alter many cell functions. Inhibitors of methylation reactions have been shown to affect cell differentiation, leukocyte chemotaxis, and virus replication. However, the specific reaction(s) involved are not clear, since most methylation inhibitors are not specific for just one reaction. The possible clinical applications could be in the development of compounds for use in chemotherapy, immunosuppression, and antiviral drugs.

Proposed Course of Research:

Studies on several inhibitors will continue in order to determine specific mechanisms of inhibition, and to determine correlations between inhibition of specific reactions and the physiological effects of these compounds. Much of the work will focus on methylation reactions involved in leukocyte chemotaxis, and in RNA and protein synthesis. The differences in inhibition of lipid and protein methylation by the various compounds will continue to be examined.

Publications:

Montgomery, J.A., Clayton, S.J., Thomas, H.J., Shannon, W.M., Arnett, G.,

Bodner, A.J., Kim, I.-K., Cantoni, G.L., and Chiang, P.K.: The carbocyclic analog of 3-deazaadenosine: a novel antiviral agent using S-adenosylhomocysteine hydrolase as a pharmacological target. J. Med. Chem. 25: 626-629, 1982.

Pritchard, P.H., Chiang, P.K., Cantoni, G.L., and Vance, D.E.: Inhibition of phosphatidylethanolamine N-methylation by 3-deazaadenosine stimulates the synthesis of phosphatidylcholine via the CDP-choline pathway. J. Biol. Chem. 257: 6362-6367, 1982.

Morita, Y., Siraganian, R.P., Tang, C.K., and Chiang, P.K.: The inhibition of histamine release and choline uptake by 5'-deoxy-5'-isobutylthio-3-deazaadenosine (3-deaza-SIBA). Biochem. Pharmacol., in press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00933-08 LGCB | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Study of Adenosylmethionine Synthetase and Enzymes of Transmethylations | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: G. L. Cantoni</td> <td style="width: 33%;">Chief, Lab. Gen. Comp. Biochem.</td> <td style="width: 33%;">LGCB NIMH</td> </tr> <tr> <td>Other: I.-K. Kim</td> <td>Visiting Fellow</td> <td>LGCB NIMH</td> </tr> <tr> <td>T. M. Caryk</td> <td>Chemist</td> <td>LGCB NIMH</td> </tr> </table> | | | PI: G. L. Cantoni | Chief, Lab. Gen. Comp. Biochem. | LGCB NIMH | Other: I.-K. Kim | Visiting Fellow | LGCB NIMH | T. M. Caryk | Chemist | LGCB NIMH |
| PI: G. L. Cantoni | Chief, Lab. Gen. Comp. Biochem. | LGCB NIMH | | | | | | | | | |
| Other: I.-K. Kim | Visiting Fellow | LGCB NIMH | | | | | | | | | |
| T. M. Caryk | Chemist | LGCB NIMH | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | | | | | | | | | | |
| SECTION Section on Proteins | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Project discontinued. | | | | | | | | | | | |

Project Description:

Project discontinued.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00934-10 LGCB |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies on the Biochemical Basis of Narcotic Drug Action | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: W. A. Klee Other: W. Byrne L. Hjelmeland K. C. Rice A. E. Jacobson C. Zioudrou J. Barker F. W. Sweat W. F. Simonds | Research Chemist Guest Worker Senior Staff Fellow Research Chemist Research Chemist Visiting Scientist Chief, Lab. of Neurophysiology Research Scientist Pharmacology Research Assoc. | LGCB NIMH LGCB NIMH DPS NICHHD LC NIADDDK LC NIADDDK LGCB NIMH LNP NINCHS LGCB NIMH NIGMS |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | |
| SECTION Section on Proteins | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 3 | PROFESSIONAL: 2 | OTHER: 1 |
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| SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Opiate receptors</u> solubilized with the detergent, <u>CHAPS</u>, are macromolecular com- plexes carrying at least two activities: reversible binding of opiate ligands, and a <u>guanine nucleotide</u> (and Na^+) <u>sensitive regulatory activity</u>. In <u>membranes</u> prepared from <u>neuroblastoma x glioma hybrid cell NG108-15 or rat striatum</u>, opiate receptors inhibit <u>adenylate cyclase</u> activity by stimulating GTP hydro- lysis catalyzed by a specific, low K_m, <u>GTPase</u>. These observations provide a general mechanism for the action of opiate receptors and for other <u>inhibitory</u> <u>receptors</u> coupled to <u>adenylate cyclase</u>. Opiate receptors have been transferred from one membrane to another by fusion techniques and are specifically labelled by the appropriate <u>affinity ligands</u>. </p> | | |

Project Description:

Over the past several years we have been engaged in the characterization of the interaction between opiate receptors and adenylate cyclase in a cultured neuronal cell line, NG108-15. The cell has a large number of opiate receptors which function to inhibit adenylate cyclase and thereby lower cAMP levels. In analogy to the addictive process, the cells become tolerant to, and dependent upon, opiates after prolonged exposure. This adaptive response is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cAMP levels in the continued presence of opiates. In the past year, we have made significant progress in our understanding of the detailed mechanism of some of these opiate actions. Moreover, work in this and other laboratories has shown that the cyclic nucleotide linked mechanism of opiate action is operative in brain as well as in the cultured cell system.

As a first step towards the purification and reconstitution of the cellular constituents involved in opiate action, we solubilized opiate receptors from several sources using the zwitterionic detergent, CHAPS. Receptors which reversibly bind opiate ligands with the appropriate specificity were isolated from membranes of NG108-15 cells, brain tissue (both rat and beef), and human placenta. Each of these receptor preparations behaves as a macromolecular complex of Stokes radius near 7 nm and contains protein as an essential constituent.

The guanine nucleotides GDP, GTP and guanosine-5'(β,γ -imido)triphosphate inhibit binding of opiates and opioid peptides to receptors solubilized from neuroblastoma x glioma NG108-15 hybrid cells. The inhibition requires sodium ions and reflects a decrease in affinity of receptors for opioid ligands. In membranes, only opioid agonist binding is decreased by guanine nucleotides, but binding of both the agonist, etorphine, and the antagonist, diprenorphine, is reduced in the case of soluble receptors. These observations are consistent with the suggestion that solubilized receptors are complexes composed of an opiate binding protein and a guanine nucleotide regulatory component. Indeed, when such preparations are subjected to gel exclusion chromatography, opiate binding activity migrates together with the guanine nucleotide regulatory protein of adenylate cyclase. Opiate inhibition of adenylate cyclase activity was shown to result from a, more direct, stimulation of GTP hydrolysis catalyzed by an inhibitory receptor coupled GTPase in neuronal membranes. Neither opiate stimulation of the GTPase nor inhibition of adenylate cyclase is observed in detergent solutions of neuronal membranes. Thus, even though receptor binding in such solutions is GTP sensitive, some component of the receptor-GTPase coupling mechanism has become limiting. A major goal of our work is the reconstitution of coupling of receptor occupancy to adenylate cyclase inhibition (and GTPase stimulation) in solubilized preparations. Functional reconstruction of the coupled activities is necessary to determine the numbers of essential constituents and the role which each component plays. A first step towards reconstitution is our recent demonstration that functional opiate receptors can be transferred, by polyethylene glycol induced fusion, from membranes of NG108-15 cells to those of other cells. In these experiments NG108-15 membranes were first treated with N-ethylmaleimide so as to inactivate the adenylate cyclase but not the receptors present. After fusion with glial or lymphocyte derived cells, opiate inhibition of adenylate cyclase in the recipient membranes can be shown. Thus, opiate receptors can interact with adenylate cyclase from several sources,

including non-neuronal ones. Furthermore, the receptors must be able to diffuse freely within the membrane so as to interact with recipient-cell machinery. We hope to be able to extend these studies to include solubilized receptor preparations.

A second long-range goal of our work in the elucidation of the detailed structure of opiate, and related, receptors. We have recently prepared, and are characterizing, an array of opiate and opioid peptide derivatives which are able to both interact normally with receptors and subsequently form covalent bonds. Some of these affinity ligands display very strong selectivity towards delta or towards mu opiate receptor subtypes. These are being prepared as radiolabelled agents and will be useful for characterization of the protein subunits of the recognition sites of opiate receptor subtypes from different sources. Preliminary experiments with one of these agents suggest that a single protein (or closely related group of proteins) is specifically labelled.

Because receptor function is a property of cell surface membranes we have developed procedures to prepare such membranes as free as possible of other types of membrane structures found in the cells. Electrophoretic analysis of such purified membranes confirms the expectation that many fewer proteins are present than are found in cruder preparations. We hope to identify the proteins associated with inhibitory receptor-adenylate cyclase complexes so as to study their modifications in various physiological and pathological states.

Significance of Biological Research to the Program of the Institute:

A major problem in biology is understanding the mechanism of signal-response coupling across cell membranes. Cells communicate with one another and with their environment largely through chemical messengers which are sensed by cell surface receptors and thereby elicit other chemical changes within the cell. The opiates, and related substances, are important transmitters of information in the nervous system. An understanding of how brain cells transmit and use such information is essential to the design of rational therapy for mental illness.

Proposed Course of Research:

We plan to continue to characterize receptors and their associated enzymatic apparatus in both structural and functional terms. The ultimate goal of our work is to purify and reconstitute the separated components of the system.

Publications:

Ahmed, M.S., Byrne, W.L., and Klee, W.A.: Solubilization of opiate receptors from human placenta. In Placenta. New York, University of Rochester, 1981, pp. 115-121.

Koski, G., and Klee, W.A.: Opiates inhibit adenylate cyclase by stimulating GTP hydrolysis. Proc. Natl. Acad. Sci. U. S. A. 78: 4185-4189, 1981.

Klee, W.A., and Streaty, R.A.: Opiate dependent dual regulation of adenylate cyclase activity in a cell-free system. In Dumont, J.E., Greengard, P., and Robison, G.A. (Eds.): Advances in Cyclic Nucleotide Research. New York, Raven Press, 1981, Vol. 14, pp. 629-635.

Byrne, W.L., and Klee, W.A.: Multiple etorphine binding proteins from brain. In Advances in Endogenous and Exogenous Opioids. Tokyo, Kodansha Ltd., 1981, pp. 71-73.

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Zioudrou, C., Varoucha, D., Loukas, S., Streaty, R.A., and Klee, W.A.: Photolabile ligands for opiate receptors. Life Sci., in press.

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|---|--|--|-----------|---------------|---------------------------|-----------|--------|--------------|-------------------------------------|---------|-------------|------------|---------|----------|--|--|--|------------|-------------------|---------|--|------------|--------------------|----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00935-15 LGCB | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Effect of Small Viruses and Their Nucleic Acids on the Biochemistry of Living Organisms | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">C. R. Merrill</td> <td style="width: 40%;">Senior Research Scientist</td> <td style="width: 20%; text-align: right;">LGCB NIMH</td> </tr> <tr> <td rowspan="3">Other:</td> <td>M. Gottesman</td> <td>Chief, Biochemical Genetics Section</td> <td style="text-align: right;">LMB NCI</td> </tr> <tr> <td>S. L. Adhya</td> <td>Geneticist</td> <td style="text-align: right;">LMB NCI</td> </tr> <tr> <td>J. Horst</td> <td>Professor, Human Genetics, University of Ulm, West Germany</td> <td></td> </tr> <tr> <td></td> <td>W. G. Nash</td> <td>Expert Consultant</td> <td style="text-align: right;">LVC NCI</td> </tr> <tr> <td></td> <td>D. Goldman</td> <td>Clinical Associate</td> <td style="text-align: right;">LCS NIMH</td> </tr> </table> | | | PI: | C. R. Merrill | Senior Research Scientist | LGCB NIMH | Other: | M. Gottesman | Chief, Biochemical Genetics Section | LMB NCI | S. L. Adhya | Geneticist | LMB NCI | J. Horst | Professor, Human Genetics, University of Ulm, West Germany | | | W. G. Nash | Expert Consultant | LVC NCI | | D. Goldman | Clinical Associate | LCS NIMH |
| PI: | C. R. Merrill | Senior Research Scientist | LGCB NIMH | | | | | | | | | | | | | | | | | | | | | |
| Other: | M. Gottesman | Chief, Biochemical Genetics Section | LMB NCI | | | | | | | | | | | | | | | | | | | | | |
| | S. L. Adhya | Geneticist | LMB NCI | | | | | | | | | | | | | | | | | | | | | |
| | J. Horst | Professor, Human Genetics, University of Ulm, West Germany | | | | | | | | | | | | | | | | | | | | | | |
| | W. G. Nash | Expert Consultant | LVC NCI | | | | | | | | | | | | | | | | | | | | | |
| | D. Goldman | Clinical Associate | LCS NIMH | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Laboratory of Pathology; Laboratory of Molecular Biology, NCI Dept. of Human Genetics, University of Ulm, West Germany | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Proteins | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">3</div> | PROFESSIONAL: <div style="text-align: center;">1</div> | OTHER: <div style="text-align: center;">2</div> | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> <div> <input checked="" type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input type="checkbox"/> (c) NEITHER </div> </div> | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> Viruses which integrate into the host cells genome and effect cellular metabolism have been investigated. Integration of the bacterial virus, <u>lambda</u> into the <u>E. coli</u> genome near the bacterial genes for galactose metabolism has been demonstrated under certain circumstances, to lead to gross discordancy of the expression of the <u>galactose</u> metabolism genes. Virally induced discordinate gene expression was shown to be due an effect on the secondary structure of a messenger, RNA. The development of techniques to detect chromosomally integrated viral genomes, viral mRNA and virally altered metabolism in host cells has encouraged us to apply these technologies to investigate human diseases. The possibility of an association between a viral genome (integrated in the chromosome) and the occurrence of diseases inherited in a dominant manner, such as familial <u>Alzheimer's disease</u> is currently being examined. </p> <p> Integration of viral related nucleotide sequences in the genomic DNA of man will be studied using <u>fibroblast cell lines</u> established from individuals belonging to a family with histologically confirmed Alzheimer's disease. </p> | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

The methodologies for studying viral genes and their expression in host cells have been developed over the past decade. We employed these methods to study a model system utilizing lambda virus and *E. coli*. In this study we showed that expression of bacterial genes can depend upon the promoter from which they are transcribed. In the *E. coli* galactose operon, expression of galE (epimerase) does not occur when the operon is transcribed from a viral promoter, when the virus is integrated into the bacterial chromosome up-stream from gal operon. After surveying, by two-dimensional gel electrophoresis, the proteins synthesized after viral induction, we have come to the conclusion that the absence of epimerase activity results from failure to translate the galE mRNA sequences into a protein.

The identification of kinase and transferase was aided by the analysis of a strain carrying a high plasmid copy number into which the gal operon had been cloned. The three products of the gal operon were readily apparent in two-dimensional gels of labelled extracts made from this strain, without D-galactose induction or prior purification. This approach, using cloned genomic fragments, should prove generally useful in identifying gene products.

The extension of these methodologies to study dominant genetic diseases, such as familial Alzheimer's disease, for viral genetic information will utilize restriction endonucleases, electrophoresis, and DNA-DNA hybridization. Human DNA from fibroblast cell lines established from skin biopsies from individuals belonging to a family with histologically confirmed Alzheimer's disease will be used. These DNAs will then be screened with radioactively labelled viral probes synthesized from the nucleic acid of selected viruses. The viruses used include human; herpes virus, adenovirus, poliovirus, measles virus, cytomegalovirus, Epstein-Barr virus and BK virus, primate; SV40, baboon type-C virus, and simian sarcoma virus, and murine; Rauscher murine leukemia virus, Moloney murine sarcoma virus and mouse mammary tumor virus. These viruses were selected for their potential to detect related sequences in the human genome. The choice of human viruses is obvious as is the selection of a few primate viruses on the basis of the evolutionary relatedness of the two species. Murine viruses are also included because of the finding of 11-28% nucleic acid homology between some primate and murine viruses.

Hybridization of a viral probe with a DNA restriction fragment may indicate the presence of virally related sequences in the cell lines. Since the fibroblasts were originally obtained from individuals comprising an extensive pedigree of familial Alzheimer's disease, the presence of chromosomally integrated viral genetic information could then be followed for genetic transmission throughout the family.

Many mammalian species contain endogenous viral information which usually remains "silent" due to either repression of the virus by the cell or integration of only a portion of the viral genome. The presence of these endogenous viral sequences in a wide range of animal species indicates their evolutionary preservation, and according to some, such genetic information might provide functions with a selective advantage to the species possessing them. These

endogenous viral genes also have the potential to be turned on by environmental agents or derepressed in an aging cell resulting in cell transformation and neoplasias or a virus-related disease. In light of these findings, it seems imperative to examine the human genome for sequences related to these viruses.

Previous studies have looked for homology between DNA extracted from a cell and the DNA of a viral probe by reassociation kinetics. Using these methods, free viral nucleic acid could not be distinguished from integrated viral sequences. However, new techniques involving restriction endonuclease digestion, Southern blotting (Southern, 1975) followed by hybridization of a viral probe will allow this project to examine whether the cellular DNA of man contains integrated virus related sequences. Additionally, the sites of integration could be examined. Integration sites would be useful as polymorphic markers in the mapping of the genetic loci on the human chromosomes. This information would also aid in the understanding of gene regulation.

A number of diseases associated tissues have been examined for the presence of human viral sequences. Poliovirus type I and type II were used to study amyotrophic lateral sclerosis (Miller et al., 1980) with negative results. Human cytomegalovirus (CMV) was used to probe DNA from the brains of patients with schizophrenia (Aulakh et al., 1981) and multiple sclerosis (Aulakh et al., 1980). Both of these hybridization analyses failed to detect any virus related genetic information complementary to the CMV probe. Herpes simplex virus (HSV) has also been used extensively to search for viral nucleotide sequences in brain tissue from patients with multiple sclerosis (Aulakh et al., 1980), idiopathic Parkinson's disease (Wetmur et al., 1979), and senile and presenile dementias of Alzheimer and Pick (Middleton et al., 1980), again with negative findings. Neoplastic tissue has also been analyzed with several viral probes. One hundred and sixty-six tumors representing about 50% of all cancer types in the United States were extracted by Wold and his colleagues (1978) and hybridized with BK virus, a human papovavirus. They were unable to detect BKV sequences in the DNA from any of the human tumors.

However, these studies were all done using liquid DNA-DNA hybridization which is not as sensitive as the techniques intended for use in this project. One study which employed Southern blotting (a technique we are using) of human tonsil DNA digested with Eco RI followed by filter hybridization with group C human adenoviruses (Green et al., 1979) found that all tonsils showed restriction fragments which might indicate adenovirus sequences integrated into tonsil DNA. Other investigations which indicate positive results include the identification of integrated hepatitis B virus DNA in a human hepatocellular carcinoma cell line (Chakraborty et al., 1980) and the detection of measles virus genome in brain tissue from patients with subacute sclerosing panencephalitis and multiple sclerosis (Haase et al., 1981).

Based on this wealth of conflicting data, it seems imperative to approach this concept with the latest techniques. Alzheimer's disease is a good candidate for such research since evidence has implicated incomplete, latent or unconventional virus infections as a possible primary pathogenetic event. Possibly the disease state is a result of age-dependent relaxation of gene repression which could lead to the expression of specific viral genes not normally expressed. This relaxation of gene control has been previously suggested in

diseases which may be associated with slow viruses.

Given that endogenous viruses have been found in a wide variety of vertebrate species, such as reptiles, birds, rats, mice, pigs, cats, and primates, it seems likely that this project will uncover the presence of some virally related nucleotide sequences in the genomic DNA of man. Detection of integrated viral genes in human DNA will be of major importance in many fields of research and will spark increased analysis of virus associations in disease states.

Significance of Biological Research to the Program of the Institute:

Molecular genetic studies will prove a key to the understanding of normal brain function and the pathogenesis of neuropsychiatric diseases. There are a number of genetic neuropsychiatric diseases for which trait-specific molecular markers are lacking and for which the pathogenesis is largely unknown. Prominent among these is familial dominant Alzheimer's disease, the major focus of this project.

Because the prevalence of severe dementia in the Northern European population of age 65 or older is 4-5% and the prevalence of milder dementia is 11-12%, more than 1 million Americans are probably afflicted with severe dementia and 3 million have mild or moderate dementia. Cognitive dysfunction in old age does not occur inevitably; it is a consequence of pathological processes. Of demented patients, 50% show the neuropathological changes of Alzheimer's disease: neurofibrillary tangles, senile plaques and granulovacuolar bodies.

The diagnosis of Alzheimer's disease is presently clinical. Other causes of chronic cognitive deterioration such as toxins, nutritional deficiencies, infections, endocrine disturbances, cerebral tumors, arterial disease and normal-pressure hydrocephalus must be ruled out. In elderly patients, depression frequently masquerades as dementia. Definitive diagnosis currently depends on the histological study of brain tissue. Brain neurochemical findings have stimulated investigators to attempt treatment by manipulating cholinergic neurotransmission and approaches might be made to the treatment of other dementias. There is a definite need for a better method for diagnosing Alzheimer's disease.

The cause of Alzheimer's disease is unknown. It may be a slow infectious agent such as scrapie in sheep or Kuru and Creutzfeldt-Jakob disease in man. Psychiatric and neurologic sequelae may follow years after viral infection (as with measles and subacute sclerosing panencephalitis and with influenza and postencephalitic Parkinson's disease). The paired helical filaments of neurofibrillary tangles and neuritic plaques in Alzheimer's disease could be due to the interaction of viral protein with cell cytoskeletal protein which occurs during viral replication. A factor from brains and Alzheimer patients assembled neurofilaments into paired helical filaments and was cytopathic in neuronal cell cultures. The factor was RNase and protease sensitive but DNase resistant. Brain suspension from patients with familial Alzheimer's disease showed cell-fusing activity in 59% of cases, a level similar to that for Creutzfeldt-Jakob disease; only 3 of 17 sporadic Alzheimer cases (17%) showed fusion activity. Like Moloney leukemia virus, the putative virus or other infectious agents might integrate into the human germ cell genome, be transmitted in autosomal dominant fashion and show tissue specific expression or effects. Alternatively, susceptibility to infection

would have both for genetic determinants. We will be searching both for viral sequences used as genetic markers and as potential causative agents.

The strongest evidence for a genetic factor in Alzheimer's disease is the fact that all individuals with Trisomy 21 develop the brain pathology of Alzheimer's disease if they live to their late 20's or 30's. Based on this fact, our laboratory has approached the molecular basis of Alzheimer's disease by attempting to identify (using 2DE) proteins coded for or modulated by Chromosome 21.

Molecular polymorphisms are genetic variants with an allelic frequency of greater than 1% in the normal population. This project will help to establish the amount of exchange there has been between the human genome and viral genomes. Integrated viral sequences are not only of interest in themselves but may often behave as polymorphisms and serve as diagnostic markers and molecular probes. When the polymorphic locus is close to a disease locus, the two loci will rarely be separated by recombination and linkage may be established. Mathematical analysis indicates that approximately 20% of the human genome is currently covered at a genetic linkage distance of 10 centimorgans. Discovery of a sufficient number of polymorphic marker loci will allow the construction of a high resolution map of the human genome. Such a map will allow most genetic diseases which are primarily monogenic to be localized to a genomic subregion and will provide additional molecular probes for investigations of pathogenesis.

Proposed Course of Research:

The work will proceed utilizing the Alzheimer's pedigree and other large pedigrees. It will be aimed towards the identification, genomic localization and fine study and testing for disease associations of viral sequences in the human genome. If this approach is promising it will be extended to other neuropsychiatric diseases.

Publications:

Merril, C.R., Gottesman, M.E., and Adhya, S.L.: E. coli gal operon proteins made after prophage lambda induction. J. Bact. 147: 875-887, 1981.

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|--|---|--|----------------|---|-----------|------------------|---|--|------------|--|--|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 00936-19 LGCB | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Homocystinuria: Methionine Metabolism in Mammals | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: S. H. Mudd</td> <td style="width: 40%;">Chief, Section on Alkaloid Biosynthesis</td> <td style="width: 30%;">LGCB NIMH</td> </tr> <tr> <td>Other: F. Skovby</td> <td>Postdoctoral Fellow, Dept. of Human Genetics, Yale University, New Haven, Conn.</td> <td></td> </tr> <tr> <td>H. L. Levy</td> <td>Assist. Prof. of Neurology and Assist. Prof. of Pediatrics, Amino Acid Lab., Massachusetts General Hospital, Boston, Mass.</td> <td></td> </tr> </table> | | | PI: S. H. Mudd | Chief, Section on Alkaloid Biosynthesis | LGCB NIMH | Other: F. Skovby | Postdoctoral Fellow, Dept. of Human Genetics, Yale University, New Haven, Conn. | | H. L. Levy | Assist. Prof. of Neurology and Assist. Prof. of Pediatrics, Amino Acid Lab., Massachusetts General Hospital, Boston, Mass. | |
| PI: S. H. Mudd | Chief, Section on Alkaloid Biosynthesis | LGCB NIMH | | | | | | | | | |
| Other: F. Skovby | Postdoctoral Fellow, Dept. of Human Genetics, Yale University, New Haven, Conn. | | | | | | | | | | |
| H. L. Levy | Assist. Prof. of Neurology and Assist. Prof. of Pediatrics, Amino Acid Lab., Massachusetts General Hospital, Boston, Mass. | | | | | | | | | | |
| COOPERATING UNITS (if any) Dept. of Human Genetics, Yale University, New Haven Conn. Amino Acid Lab., Massachusetts General Hospital, Boston, Mass. | | | | | | | | | | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | | | | | | | | | | |
| SECTION Section on Alkaloid Biosynthesis | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | |
| TOTAL MANYEARS: 5/12 | PROFESSIONAL: 5/12 | OTHER: 0 | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) An international <u>questionnaire survey</u> aimed at obtaining standardized information to provide insight into many presently unanswered questions as to <u>clinical features of homocystinuria</u> due to <u>cystathionine β-synthase deficiency</u> and the <u>effects of various therapies</u> now in use for this condition is underway. Based upon present results and upon projected responses, it appears that by completion of data collection in early 1983 <u>reports will have been obtained upon at least 325 patients</u> , perhaps more. This should be sufficient to provide statistically meaningful answers to the questions raised. | | | | | | | | | | | |

Project Description:

Within the general framework of investigating methionine metabolism in mammals, with emphasis on the human genetic diseases which results from abnormalities affecting this area of metabolism, efforts this year have focussed upon a questionnaire survey of patients with homocystinuria due to cystathionine β -synthase deficiency. The purpose of the present survey is to gather sufficient information in a standardized format so that some of the major uncertainties about the natural history of this disease, about the role of genetic heterogeneity, and concerning the use and effectiveness of various therapies may be assessed. Homocystinuria was first described in 1962 and cystathionine β -synthase deficiency in these patients was demonstrated in 1964. Once the enzyme defect had been identified, it became possible to devise dietary therapy which, in theory, might be expected to prevent or mitigate the clinical consequences of this condition. In 1967 it was found that some, but not all, patients respond biochemically to large doses of vitamin B₆ (usually given in the form of pyridoxine). Subsequently it has become clear that the capacity to respond, or not to respond, to B₆ is also genetically determined and reflects subtle differences in the genetic lesions which bring about cystathionine β -synthase deficiency. Since 1964 (or 1967) some, perhaps most, patients with this condition have been given trials or prolonged periods of dietary and/or pyridoxine therapy, but, because of the very serious sequelae of the disease, this has been carried on in the absence of any control, untreated population. More recently, certain "antiplatelet" drugs designed to prevent thrombosis have been recommended for therapy, and are now being used to an undetermined extent.

Because a relatively short time elapsed between discovery of homocystinuria and initiation of the use of various therapies, historically there was little chance to accumulate knowledge about the natural course of the condition in untreated patients. It has become clear that the time of onset and the severity of the clinical manifestations may vary widely from one patient to another. This combination of circumstances, in retrospect, make it extremely difficult at the present time to assess many aspects of the natural course of the untreated disease (taking proper account of genetic heterogeneity). Among the uncertainties are: the time of onset and the frequency of the major complications, mental retardation, bony deformities, and dislocated optic lenses; the frequency and incidence of cardiovascular accidents; the impact of maternal homocystinuria upon reproductive success and fetal well-being; the factors that bring these patients to medical attention; the proportion of patients who are being missed by screening programs of the newborn intended to ascertain individuals early enough to start effective preventive therapy. Further, there is no accurate information as to what therapies are now actually being used. Together, these problems prevent realistic assessment of the impact of various therapies. Until such an assessment is made, it is difficult rationally to discontinue ineffective therapies which nevertheless have their own adverse side-effects, and to continue and improve those which are effective. These difficulties are all compounded by the fact that homocystinuria due to cystathionine β -synthase deficiency is a relatively rare disease. In screening programs of new-born children now in use in the United States, Western Europe, and Japan about 1:200,000 neonates are found to have this condition. About 300 patients, with this enzyme deficiency, have been reported in the literature worldwide. We estimate another 200 have been identified. These patients have been cared for probably by about 150 physicians.

No single physician or medical center has been able to accumulate a large enough sample of patients to answer the problems outlined above.

The plan of the present survey is to obtain from physicians caring for homocystinuric patients responses to questionnaires dealing with certain aspects of the natural history and therapy of such patients. To this end, a questionnaire was developed in consultation with several physicians who themselves are expert in homocystinuria and are responsible for large numbers of patients. An OMB clearance (#0930-0075) was obtained in the fall of 1981. In January, 1982, approximately 800 questionnaires were disbursed to physicians in all parts of the world. The predominant portion of these physicians were known to have treated or published upon one or more homocystinuric patients, but many questionnaires were sent to physicians in academic or diagnostic centers in hopes of ascertaining patients of whom we were not aware. Since then, about 300 additional questionnaires have been disbursed as further ideas and suggestions were received as to appropriate physicians.

Significance of Biomedical Research to the Program of the Institute:

The emphasis in this project has been upon human genetic diseases due to defects in the metabolism of sulfur-containing compounds. Many of these diseases lead to mental retardation; understanding their etiology and pathophysiology may be expected to help in prevention of such retardation and of other serious manifestations. Work on inborn errors has repeatedly been shown to illuminate normal human biochemistry and enzymology, and study of the diseases in question have likewise proven useful in furthering understanding of normal human metabolism.

Proposed Course of Research:

The response to date has been encouraging. We have in hand reports covering more than 240 patients. Verbal and written responses concerning additional patients allow a reasonable projection that as many as 325, perhaps 400, responses will ultimately be obtained. Our plan is to try to finish almost all data collection during calendar 1982, although completion of responses is regarded as preferable to terminating the project by any fixed and arbitrary date. Follow-up letters and, if need be, telephone calls to responding physicians are planned for the near future.

Data processing has not yet begun, but arrangements for data processing by computer analysis have been made through Mr. Emmett Ward, Chief Data Management Branch, NIH. Ms. Karen Pettigrew, Statistical Consultant to the Intramural Research Program, of the National Institute of Mental Health, has been consulted and has approved statistical aspects of the questionnaire and the plan for analysis of the data. Both these individuals will be available for further consultation and advise as problems arise during the actual processing of the data. During July, 1982, entry of the available data into suitable computer form will commence with the assistance of Dr. Fleming Skovby, Dept. of Human Genetics, Yale University.

Dr. Skovby will spend short periods at NIH during the remainder of his fellowship at Yale, which terminates in December, 1982, and has made plans to spend 2-3

months here in January-March or February-April, 1983, to complete data processing and hopefully prepare an initial draft of a manuscript. Arrangements for Dr. Skovby's appointment as a Visiting Associate are now underway. If all goes well, these arrangements, together, will permit completion of the survey project and preparation of a manuscript approximately by mid-1983. At the moment it appears clear that a sufficiently large number of completed patient reports should be received so that there are real possibilities for gaining statistically meaningful answers to the questions raised above.

Publications:

Mudd, S.H., Havlik, R., Levy, H.L., McKusick, V.A., and Feinleib, M.: Lack of excess heart attack or stroke risk in heterozygotes for homocystinuria due to cystathionine synthase deficiency. Am. J. Hum. Genet. 33: 883-893, 1981.

Mudd, S.H., and Levy, H.L.: Disorders of Transsulfuration. In Stanbury, J.B., Wyngaarden, J.B., and Fredrickson, D.S. (Eds.): The Metabolic Basis of Inherited Disease, 5th Ed. New York, McGraw-Hill, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00937-17 LGCB |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Transsulfuration in Higher Plants | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: J. Giovannelli Other: S. H. Mudd A. H. Datko G. A. Thompson | Research Chemist Chief, Section on Alkaloid Biosynthesis Botanist Senior Staff Fellow | LGCB NIMH LGCB NIMH LGCB NIMH LGCB NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | |
| SECTION Section on Alkaloid Biosynthesis | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Project discontinued. | | |

Project Description:

Project discontinued.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00939-02 LGCB | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Enzymes of Methionine Biosynthesis in Higher Plants | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: G. A. Thompson</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LGCB NIMH</td> </tr> <tr> <td>Other: A. H. Datko</td> <td>Botanist</td> <td>LGCB NIMH</td> </tr> <tr> <td>S. H. Mudd</td> <td>Chief, Section on Alkaloid Biosynthesis</td> <td>LGCB NIMH</td> </tr> </table> | | | PI: G. A. Thompson | Senior Staff Fellow | LGCB NIMH | Other: A. H. Datko | Botanist | LGCB NIMH | S. H. Mudd | Chief, Section on Alkaloid Biosynthesis | LGCB NIMH |
| PI: G. A. Thompson | Senior Staff Fellow | LGCB NIMH | | | | | | | | | |
| Other: A. H. Datko | Botanist | LGCB NIMH | | | | | | | | | |
| S. H. Mudd | Chief, Section on Alkaloid Biosynthesis | LGCB NIMH | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry SECTION Section on Alkaloid Biosynthesis | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | |
| 1-6/12 | 1-6/12 | 0 | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> An investigation of the effects of sub-lethal doses of inhibitors of <u>methionine biosynthesis</u> upon the growth and morphology of <u>Lemna (duckweed)</u> has demonstrated that these plants <u>adapt</u> to conditions of limiting methionine. Adaptation is evidenced by a transient period between 24 and 48 hours after initial exposure when individual colonies break apart abnormally and no new fronds appear, followed by resumption of frond emergence, restoration of a normal frond/colony ratio, and increased tolerance to the same or higher concentrations of the inhibitor. Investigation of wet weight and protein sulfur accumulation during exposure to 40 nM <u>propargylglycine</u> (PAG) showed that neither of these parameters undergoes a parallel transient effect, but instead slows to the "adapted" steady-state rate by 24 hours after first exposure. Studies of <u>cystathionine γ-synthase</u> activity during and after adaptation to PAG and/or <u>lysine plus threonine</u> suggest that adaptation is related, in part, to increases in the steady-state concentrations of this and other enzymes in the methionine pathway. The relationship between these changes and the morphological effects remains unclear. </p> | | | | | | | | | | | |

Project Description:

A major objective of research in this laboratory is to elucidate the mechanism(s) of control of methionine biosynthesis in higher plants. For these studies, specific inhibitors of enzymes catalyzing various steps in the pathway were developed. When these inhibitors are administered to our experimental model plant, Lemna paucicostata (duckweed), the reduced capacity of the plant to make methionine becomes growth limiting. Under these limiting conditions the activity of the enzyme which catalyzes the committing step toward methionine, cystathionine γ -synthase, becomes dramatically increased (see previous annual report, Z01 MH 00937-15, 1980). The activity begins to increase sometime after 6 hours of exposure and continues to increase for up to 72 hours.

Two of these inhibitory regimens, propargylglycine (PAG) and lysine plus threonine have been extensively studied in this laboratory. PAG inhibits cystathionine γ -synthase, while lysine plus threonine synergistically block the formation of homoserine from aspartate. The net result of either regimen is to prevent synthesis of cystathionine from O-phosphohomoserine and cysteine.

During the course of our studies with PAG and lysine plus threonine, it was observed that when given growth-limiting but sub-lethal doses of inhibitor(s), the plants underwent changes which were adaptive in nature. Initially a drop in frond/colony ratio took place due, in part, to dissociation of colonies into abnormal one-or two-fronded colonies. By 24 hours after administration of inhibitor(s), frond emergence virtually halted. However, by 48 hours, frond emergence resumed (at a somewhat slower rate than normal), accompanied by a gradual rise in frond/colony ratio. By 72 to 96 hours, steady-state growth was re-established. Thereafter, the plants withstood subculture into identical concentrations of the inhibitor(s) without fragmentation or a lag in growth and demonstrated enhanced tolerance to higher concentrations of the inhibitor(s).

We undertook an investigation of the factors involved in these phenomena, which revealed the following:

- (1) Individual colonies, regardless of their stage in the life cycle of the plant, each undergo similar effects. Thus, the adaptation is not the result of outgrowth of certain especially resistant plants.
- (2) Addition of exogenous methionine prevents these effects, and overcomes a PAG-induced transient lag in frond emergence even after it is underway.
- (3) The rate of accumulation of protein sulfur and wet weight adjusts to the adapted steady-state rate by 24 hours after initial exposure to 40 nM PAG. There is no transient lag in either case. Thus, during the period between 24 and 48 hours when new fronds are not appearing, the protein sulfur and wet weight per frond is increasing.
- (4) When 40 nM PAG is administered, the soluble PAG inside the plants increases during the first 48 to 72 hours of exposure and then levels off. Thus, the internal concentration of PAG continues to increase until after frond emergence has resumed.

(5) During initial exposure to 40 nM PAG, cystathionine γ -synthase activity declines to a nadir of 9% of control after 12 hours and then climbs back to a steady-state level of 12% of control. This finding is consistent with the previously demonstrated tendency of cystathionine γ -synthase activity to increase when methionine becomes growth limiting and is a reasonable resultant of offsetting increases in the concentrations of both the enzyme and its inhibitor. The increase in activity from 9 to 12% of control is not insignificant. Whereas 9% of control activity is insufficient to sustain viability, 12% of control activity is sufficient to permit growth at 75% of the normal rate.

(6) When exposed to 36 μ M lysine plus 3 μ M threonine, Lemna undergoes adaptation after which it has 1.8 times the normal amount of cystathionine γ -synthase activity. However, when PAG is given to plants growing under these conditions, it was found that a normal growth rate could be maintained with only 50% of normal synthase activity. Therefore, the observed increase in cystathionine γ -synthase activity is not the primary factor involved in adaptation to lysine plus threonine.

Together, these results suggest that the plants respond to methionine limitation within 12 to 24 hours by increasing the concentrations of cystathionine γ -synthase and probably other enzymes in the methionine pathway, thereby enabling the plant to re-establish a steady-state rate of protein and wet weight growth. The accompanying effects on frond emergence and colony fragmentation remain unexplained. Perhaps they are related to depletion of a derivative of methionine which takes longer to establish a new steady-state concentration. Experiments in which plants were supplemented with sufficient exogenous spermidine or choline to meet their growth requirements both before and during exposure to 40 nM PAG showed that spermidine (but not choline) prevents the lag in frond emergence and colony fragmentation. Because exogenous spermidine fed to control plants causes increases in the concentrations of all proteins, including cystathionine γ -synthase, interpretation of these results with spermidine requires further study.

Significance of Biomedical Research and to the Program of the Institute:

This project is part of our general program to investigate the aspartate biosynthetic pathway in higher plants. The general significance of this research has been set forth in the report on the "Pathways of methionine and threonine metabolism, and their control, in higher plants," by Dr. Giovannelli.

Proposed Course of Research:

During the next year we plan to continue our studies by:

(1) Using two-dimensional gel electrophoresis to study changes in the amounts and turnover rates of cystathionine γ -synthase and other enzymes related to methionine metabolism (e.g. aspartokinase, homoserine dehydrogenase, threonine synthase, β -cystathionase, methionine adenosyltransferase) in Lemna growing either in the presence of external methionine or with inhibitors which bring about methionine deprivation. Preliminary experiments have resulted in two-dimensional gels with hundreds of protein spots. Thus, partial purification of

each enzyme to be studied will be helpful in locating its position on the gel. Partial purifications of cystathionine γ -synthase and threonine synthase have already been accomplished. The availability of purified enzymes would also allow us to investigate physico-chemical and allosteric properties relevant to regulation.

(2) Investigating the molecular mechanism involved in the increase in cystathionine γ -synthase activity when methionine is limiting. Questions to be examined include: (a) Does cystathionine γ -synthase activity increase when the conversion of methionine to S-adenosylmethionine is blocked by specific inhibitors of methionine adenosyltransferase? A positive answer to this question would indicate involvement of a derivative of methionine related to S-adenosylmethionine in the regulation of cystathionine γ -synthase. (b) Do inhibitors of protein synthesis prevent synthesis of cystathionine γ -synthase and thereby block the increase in activity observed under conditions of limiting methionine? A positive answer to this question would support our other evidence that the changes in activity are the result of changes in protein synthesis and/or degradation rather than some form of allosteric control. (c) What happens to cystathionine γ -synthase activity when Lemna becomes limited for both methionine and cysteine such as would be the case when faced with inadequate supplies of $\text{SO}_4^{=}$? Results from these studies might indicate whether cysteine is involved as a secondary factor in the regulation of methionine synthesis.

Publications:

Thompson, G.A., Datko, A.G., Mudd, S.H., and Giovannelli, J.: Methionine biosynthesis in Lemna: Studies on the regulation of cystathionine γ -synthase, O-phosphomoserine sulfhydrylase, and O-acetylserine sulfhydrylase. Plant Physiol. 69: 1077-1083, 1982.

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|---|---|--|-----------------|----------|-----------|------------|---|-----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00940-02 LGCB | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Methionine Biosynthesis in Higher Plants | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: A. H. Datko</td> <td style="width: 40%;">Botanist</td> <td style="width: 30%;">LGCB NIMH</td> </tr> <tr> <td>S. H. Mudd</td> <td>Chief, Section on Alkaloid Biosynthesis</td> <td>LGCB NIMH</td> </tr> </table> | | | PI: A. H. Datko | Botanist | LGCB NIMH | S. H. Mudd | Chief, Section on Alkaloid Biosynthesis | LGCB NIMH |
| PI: A. H. Datko | Botanist | LGCB NIMH | | | | | | |
| S. H. Mudd | Chief, Section on Alkaloid Biosynthesis | LGCB NIMH | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | | | | | | | |
| SECTION Section on Alkaloid Biosynthesis | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, MD 20205 | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">9/12</div> | PROFESSIONAL: <div style="text-align: center;">9/12</div> | OTHER: <div style="text-align: center;">0</div> | | | | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input checked="" type="checkbox"/> (c) NEITHER </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div> <input type="checkbox"/> (a1) MINORS </div> <div> <input type="checkbox"/> (a2) INTERVIEWS </div> </div> | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The <u>uptake of sulfate</u> by <u>Lemna</u> has been studied. Two systems have been demonstrated. One has <u>high affinity</u> for sulfate (K_m approx. 9 μM) and a V_{max} of 2.7 nmole/frond x day. This system is dramatically <u>down-regulated</u> by prior growth of <u>Lemna</u> in either <u>cystine</u> or high concentrations of <u>sulfate</u>. A second uptake system has an affinity for sulfate so <u>low</u> as not to be saturable at tolerable concentrations of sulfate (up to 25 mM). At 25 mM sulfate, the uptake via this system is 7-10 nmole/frond x day. This system is down-regulated little, if at all, by prior growth of the plants in cystine or high concentrations of sulfate. Neither system is inhibited by phosphate or nitrate at the concentrations in normal growth medium. The <u>temperature dependence</u> of the high affinity system is more marked than is that of the low affinity system. </p> | | | | | | | | |

Project Description:

For several years, the chief aim of this project has been to provide information upon the biology and physiology of Lemna (duckweed), the plant that is currently being used almost exclusively in this laboratory as the experimental system in which to study methionine biosynthesis and its regulation in higher plants. The many unusual, perhaps unique, advantages of Lemna for biochemical experimentation have been detailed in previous annual reports. Earlier investigations have resulted in the development of standardized culture conditions for Lemna suitable for biochemical work, described the life cycle of Lemna growing under these conditions, found means of growing Lemna in steady states at limiting sulfate concentrations, described a number of inhibitors which may be used to block steps in the methionine biosynthetic pathway, and demonstrated both the specificity and the extent of the resulting metabolic inhibitions.

During the past year, only a limited amount of time has been available for this project. Preliminary measurements of the effects of exogenous methionine upon growing Lemna had revealed that, among other actions, 2 μM methionine produces a 50% decrease in the uptake of sulfate by these plants. The magnitude of this decrease is such that it almost exactly compensates for the decreased need for sulfate which results from the decrease in methionine synthesis produced by exogenous methionine. To further understand this regulatory effect of methionine, a study of sulfate uptake by Lemna, and its regulation, was undertaken. To date, the chief results are the following:

- a) Studies of uptake as a function of external $\text{SO}_4^{=}$ concentration have revealed that at least two systems are involved in the uptake of this anion. One has a high affinity for sulfate, with an apparent K_m of approximately 9 μM . The system is saturable with substrate, and has a V_{max} 2.7 nmole sulfate/frond x day. The second system has an affinity so low that it is not saturated at sulfate concentrations as high as 25 mM, the upper limit of sulfate concentrations which Lemna will tolerate without growth inhibition. V_{max} and K_m of this system have therefore not been determined. At 25 mM $\text{SO}_4^{=}$ it contributes an uptake of 7-10 nmole sulfate/frond x day.
- b) The pH dependency of these systems has been determined. Minor differences were observed, but these were not sufficient to permit assay of the low affinity system under conditions such that the high affinity system was inactive. Therefore, to date all assays of the low affinity system must be corrected for the simultaneous contribution of the high affinity system.
- c) Competitive studies have shown that neither system is inhibited by the concentrations of phosphate or nitrate ions present in normal growth medium.
- d) The temperature dependence of the high affinity system is more marked than that of the low affinity system.
- e) The high affinity system is dramatically down-regulated by prior growth of the plants in cystine (for example, 80% decrease by growth in the presence of 7 μM cystine). Growth in high concentrations of sulfate also down-regulates (for example, 80% decrease by growth in the presence of 5 mM $\text{SO}_4^{=}$; 99% decrease by growth in 25 mM $\text{SO}_4^{=}$).

f) The low affinity system is down-regulated little, if at all, by growth in the presence of cystine (up to 31 μ M) or sulfate (up to 25 mM).

Together, these results support the conclusion that, at the concentration of sulfate used in our standard growth medium, uptake of this anion is mediated almost entirely by the high affinity system. The low affinity system contributes significantly only at much higher sulfate concentrations. Whereas the high affinity system is down-regulated when the plants are provided with cystine, or with unusually high sulfate concentrations, the low affinity system is not. The properties of the latter system suggest that it may largely represent diffusion-mediated uptake against which the plant has not developed means to completely protect itself.

To further investigate the regulatory processes at work, experiments have been carried out in which Lemna plants were grown in the presence of $^{35}\text{SO}_4^{=}$, ^{35}S -cystine, ^{35}S -methionine, $^{35}\text{SO}_4^{=}$ and ^{35}S -cystine of the same specific activity, or $^{35}\text{SO}_4^{=}$ and ^{35}S -methionine of the same specific activity. Growth was carried out at a range of concentrations of sulfate, cystine, and methionine and was continued long enough to attain virtual isotopic steady-state labeling. Analyses of ^{35}S -labeled compounds from these plants may be expected to reveal the steady-state concentrations of sulfate, soluble cyst(e)ine, soluble methionine, glutathione, S-methylmethionine, and S-adenosylmethionine while the plants are growing at a range of concentrations of the specified sulfur sources. Correlation of these results with concurrent regulatory effects on sulfate uptake (as well as other regulatory effects on various enzymes under study in this laboratory) may help to elucidate which compounds actually mediate these effects, to help understand the metabolic results of such regulatory events, and possibly to suggest additional sites of regulation. These analyses are currently underway and are expected to be completed within a few months.

Significance of Biomedical Research and to the Program of the Institute:

This project is part of our general program to investigate the aspartate biosynthetic pathway in higher plants. The general significance of this research has been set forth in the report on the "Pathways of methionine and threonine metabolism, and their control, in higher plants," by Dr. Giovannelli.

Proposed Course of Research:

For future work, depending in large part upon the results of the analyses just described, it may become necessary to explore, at least in brief, the interactions between sulfate and cystine in down-regulating the high affinity uptake system. Do these compounds act independently, synergistically, or through conversion to some common compound? Answers to these questions would clarify long-standing uncertainties as to the regulation of sulfate uptake by higher plants.

Two-dimensional gel electrophoresis is currently being utilized in collaboration with Dr. Thompson to clarify the molecular mechanism by which methionine up- and down-regulates cystathionine γ -synthase. If this approach is successful, we may attempt to extend it to the regulation of sulfate uptake. Are the regulatory

events under study due to changes in the number of permease-like molecules, or to changes in their functional properties?

Two additional lines of future work were indicated in last year's annual report. There has been insufficient time and manpower to commence work along these lines, but the proposals remain valid and, it is hoped, will be worked upon in the future:

(a) There is reason to believe, both on enzyme-mechanistic grounds and from the nutritional results, that propargylglycine and aminoethoxyvinylglycine (AVG) may be affecting sites other than those revealed by studies to date. AVG is known to also inhibit ACC synthase, the enzyme which converts S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of the plant hormone, ethylene. Experiments are planned to examine if this, or other pathways are inhibited by this compound.

(b) A search will be made for inhibitors which interfere with the conversion of methionine to S-adenosylmethionine. The latter compound has been proposed to be a major effector in the control of methionine biosynthesis in plants. Several compounds known to inhibit S-adenosylmethionine synthesis in other tissues will be examined for their effects on Lemna growth. Appropriate uptake and labeling experiments will be carried out in conjunction with these nutritional experiments.

Finally, it is becoming clear that a major limitation in our comprehension of the workings of the aspartate biosynthetic pathway in higher plants is due to lack of knowledge about the subcellular localization of the enzymes of the pathway. We plan to initiate attempts to isolate mitochondria, chloroplasts, and microsomes from Lemna in suitable form so that the subcellular distribution of relevant enzymes can be determined.

Publications:

Datko, A. H., and Mudd, S.H.: Methionine biosynthesis in Lemna: Inhibitor studies. Plant Physiol. 69: 1070-1076, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00941-02 LGCB | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Biochemical Genetics and Metabolic Disease | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: C. R. Merrill</td> <td style="width: 40%;">Senior Research Scientist</td> <td style="width: 30%;">LGCB NIMH</td> </tr> <tr> <td>Other: M. H. Ebert</td> <td>Clinical Director</td> <td>NIMH</td> </tr> <tr> <td>G. C. Salmiraghi</td> <td>Associate Director for Research</td> <td>NIAAA</td> </tr> <tr> <td>D. Goldman</td> <td>Clinical Associate</td> <td>LCS NIMH</td> </tr> <tr> <td>M. Miller</td> <td>Biochemist</td> <td>DCCP NCI</td> </tr> <tr> <td>D. Jacobowitz</td> <td>Neurochemist</td> <td>LCS NIMH</td> </tr> <tr> <td>S. O'Brien</td> <td>Chief, Section on Genetics</td> <td>LVC NCI</td> </tr> <tr> <td>W. S. Rasband</td> <td>Computer Systems Analyst</td> <td>IRP NIMH</td> </tr> </table> | | | PI: C. R. Merrill | Senior Research Scientist | LGCB NIMH | Other: M. H. Ebert | Clinical Director | NIMH | G. C. Salmiraghi | Associate Director for Research | NIAAA | D. Goldman | Clinical Associate | LCS NIMH | M. Miller | Biochemist | DCCP NCI | D. Jacobowitz | Neurochemist | LCS NIMH | S. O'Brien | Chief, Section on Genetics | LVC NCI | W. S. Rasband | Computer Systems Analyst | IRP NIMH |
| PI: C. R. Merrill | Senior Research Scientist | LGCB NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| Other: M. H. Ebert | Clinical Director | NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| G. C. Salmiraghi | Associate Director for Research | NIAAA | | | | | | | | | | | | | | | | | | | | | | | | |
| D. Goldman | Clinical Associate | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| M. Miller | Biochemist | DCCP NCI | | | | | | | | | | | | | | | | | | | | | | | | |
| D. Jacobowitz | Neurochemist | LCS NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| S. O'Brien | Chief, Section on Genetics | LVC NCI | | | | | | | | | | | | | | | | | | | | | | | | |
| W. S. Rasband | Computer Systems Analyst | IRP NIMH | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) National Institute on Alcohol Abuse and Alcoholism; Laboratory of Clinical Science, NIMH; Division of Cancer Cause and Prevention and Laboratory of Viral Carcinogenesis, National Cancer Institute | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Proteins | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIMH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 3 | PROFESSIONAL: 1 | OTHER: 2 | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project's primary goal is the development of diagnostic markers and mo- lecular clues for the understanding of <u>genetic diseases</u> of the central nervous system. A major effort has been placed on the use of <u>two-dimensional electro-</u> <u>phoresis (2DE)</u> to separate proteins from complex solutions coupled with the use of <u>silver staining</u> , <u>autoradiography</u> , and automated computer densitometry. We have been able to: identify primary mutational events, detect secondary effects of a primary mutation (quantitative alterations in eleven <u>lymphocyte</u> proteins in the <u>Lesch-Nyhan syndrome</u>), and discover <u>polymorphisms in 2DE patterns</u> of human lymphocyte proteins. The ability to observe multiple polymorphisms in a subset of lymphocyte proteins with 2DE raises the possibility that this tech- nique may provide a powerful tool for human <u>genetic linkage studies</u> . Genetic modelling studies predict that 200 polymorphisms would provide a human genetic linkage map which will prove useful in disease studies. A large family with <u>familial Alzheimer's disease</u> will be studied with these methods. This study should provide for the identification of additional polymorphisms and may un- cover disease <u>trait specific markers</u> . | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Accurate classification and understanding of genetic diseases largely depends on the detection of molecular variants and disease-associated markers. The number of genetic disease markers will increase at a great rate over the next few years due to the application of new methodologies.

A brief review of this project's approach to developing molecular probes is given in this report. Major stress is placed on two-dimensional electrophoresis (2DE) of proteins and our experiences with this approach.

Methods for identification of proteins affected by primary mutational events have evolved and proliferated over the past three decades, since the work of Pauling and his co-workers on sickle cell anemia. One of the newest methods, two-dimensional electrophoresis, can separate a thousand proteins from a single tissue on the basis of their isoelectric point and mass. We have utilized 2DE to demonstrate a primary mutation in the β -actin gene in a human fibroblast strain. We were also able to counter a claim that the enzyme hypoxanthine phosphoribosyltransferase is abnormal in the Gilles de la Tourette syndrome.

At another level, molecular probes may prove useful in detecting and cataloging secondary effects of a primary mutation. These effects are often due to feed-back control mechanisms, post-translational modifications, or altered protein stabilities.

In the Lesch-Nyhan syndrome, in which the primary defect is a deficiency of hypoxanthine phosphoribosyltransferase activity, five other enzymes have been found to be altered. The specific activities of erythrocyte adenine and inosinic acid dehydrogenase are elevated, while erythrocyte orotate phosphoribosyltransferase, orotate glutamine amidotransferase activities are also increased. Use of 2DE revealed eleven quantitative differences in lymphocytes (in a survey of 400 proteins in each lymphocyte 2DE). The differences were present in the patterns of all of the Lesch-Nyhan patients examined and were not found in any normal individuals. Secondary protein modulations have also been observed in fibroblasts with altered numbers of chromosome 21, cells exposed to hormones, or heat shock, and cells which have undergone neoplastic transformation.

Expression of some proteins is tissue-specific and currently, only a fraction of the cellular proteins are analyzed, even with 2DE. A primary defective protein may not be present in the fraction analyzed or the mutational event may not produce a detectable alteration by 2DE. However, specific secondary metabolic effects may be present and serve as an aid in the diagnosis and understanding of the pathophysiology of metabolic diseases. A correlative catalog of these effects may provide significant new insights when various inborn errors with similar phenotypic expression are compared.

Human genetic diseases may be studied by linkage analysis. This approach requires the development of a genetic map of the human genome. This map may be related to the physical map of the chromosome. As an example of this approach, a child with mental retardation and bilateral retinoblastoma was found to have a deletion in the long arm of Chromosome 13. This linkage of a deletion in Chromosome 13 and retinoblastoma and other abnormalities was later found in

family studies of a number of other cases.

Recently, the use of polymorphic markers, including blood groups, histocompatibility antigens, and electrophoretically polymorphic proteins has been found to be useful for establishing genetic linkage. Polymorphisms are genetic variants which occur with an allelic frequency of 1% or more. Highly polymorphic regions may, in general, be regions which have less constraint for a precise DNA sequence. In this regard, DNA polymorphism studies have demonstrated greater variation in intervening sequences than in coding sequences, while variations in the hemoglobins are the same in carp as man despite their evolutionary distance. As a final example, the excised portion of proinsulin varies more than the functional portions.

A number of laboratories are developing, by recombinant techniques, random single copy DNA probes for the detection of DNA restriction fragment length polymorphisms (RFLPS). Discovery of a sufficient number of RFLPS will permit construction of a high resolution genetic linkage map.

This endeavor may be complimented by use of protein 2DE, as in this project, 2DE has the capacity to screen large numbers of polymorphisms. Polymorphic variants which have been described using 2DE include serum haptoglobin, Gc-globulin, α -1 antitrypsin, α -2HS glycoprotein, arginine-rich lipoprotein, and the unidentified serum protein G-4, a polymorphic protein of brain, PC-1 Duarte, and two lymphocyte proteins which show genetic transmission. Furthermore, 2DE resolved 13 of 17 isoenzymes produced from five loci.

Discovery of a sufficient number of polymorphisms will ensure successful linkage analysis for diseases which are primarily monogenic. Barriers to the discovery of a sufficient number are technical, not theoretical. Polymorphisms of proteins and of DNA sequence are present in enormous numbers. Harris found that 24 of 104 human enzyme loci were polymorphic in a European caucasian population, for an average heterozygosity of 6.3%. Based on the observed frequency of protein polymorphism and the approximation that one third of base pair alterations produce a change in protein charge, a lower limit estimate for DNA sequence polymorphism is 0.001/base pair.

Detection of protein polymorphisms with quantitative 2DE was evaluated in a study of lymphocyte proteins from 28 individuals. One hundred and eighty-six of the most dense polypeptide spots from each autoradiogram of ^{14}C -labeled phytohemagglutinin-stimulated lymphocytes were measured for variation in position or density. Seven probable polymorphisms were identified among six of these proteins for a calculated minimum average heterozygosity of 1.2%. Five proteins had heterozygosities of greater than 0.36, indicating that many rarer polymorphic variants will be detected, even in the small subset of visualized lymphocyte proteins we studied. These protein variations were diagnosed as probable polymorphisms because they met the following criteria: 1) all variants displayed charge alterations, 2) a phenotypic heterozygous state was observed for each variant in which two proteins could be observed, each with 50% of quantity of the protein found in the homozygous state, 3) two sets of monozygotic twins shared the same phenotype for each polymorphism, 4) at least three phenotypic (two homozygous and a heterozygous) states were observed for each polymorphism. Confirmation of these polymorphisms will depend on observation of genetic trans-

mission in family studies which are currently in progress.

The ability to observe multiple polymorphisms in a subset of the proteins visible on the lymphocyte electrophoretogram raises the possibility that 2DE may provide a powerful tool for human genetic linkage studies. Because more than 1000 polypeptides may be visualized, we estimated from this study that 40 common and 200 more rare polymorphisms could be rapidly scored on each electrophoretogram. Sixty-three might be observed to be in the heterozygous state in any individual.

Polymorphic proteins may be mapped to chromosomal locations by analysis of proteins in hybrid cells with a single human chromosome (as compared to those in the non-human parent cell), by looking for gene dosage effects in cell lines with varying ploidy of a chromosome, or by using an antibody prepared against a single protein to isolate a protein mRNA complex. This mRNA can be transcribed into cDNA with reverse transcriptase. The cDNA could be used as a DNA polymorphism probe and could also determine chromosomal location by in situ hybridization.

The polymorphisms now generally available for linkage studies are detected by one-dimensional electrophoresis and serologically. Since they number less than 50, approximately 20% of the genome is covered at a 10 centimorgan linkage distance. The length of the human genome is 3300 centimorgans (cM). Our genetic modelling has shown that 200 randomly located polymorphisms would cover almost 70% of the genome. Analysis of 1000 proteins per tissue 2-D electrophoretogram may allow more than 40 commonly polymorphic proteins and 200 rarer ones to be rapidly evaluated on a single 2DE gel. Other tissues, such as fibroblasts and erythrocytes and the use of subcellular fractions will provide many additional protein markers for linkage analysis. When a polymorphic locus is close to a genetic disease locus, the two loci will rarely be separated by recombination and the polymorphism may be useful as a marker and to locate the disease locus. A collaborative effort with Dr. D. Jacobowitz, LCS, NIMH, is currently underway to develop a protein map for micro-regions of the central nervous system. Such a map will be useful for pharmacology as well as pathology studies. Dr. Harris (Glasgow, Scotland) will be joining us in the fall to use these techniques in a study of human spinal fluid proteins in normal and disease states.

One of the new methodologies which has facilitated these studies is the use of ultra-sensitive silver stains for polymer detection. The use of silver as an ultra-sensitive protein stain came about after an extensive search for new protein stains was undertaken in our laboratory. In August of 1978, Drs. Merrill and Shifrin tried heavy metal stains (such as uranium and lead), fluorescent stains (including fluorescamine and 2-methoxy-2,4-diphenyl-3-(2H)-furanone (MDPF), Hoffman La Roche, NJ) and chemiluminescent stains. Sensitivities of these stains was less than or at best, equivalent to Coomassie blue. It wasn't until the application of a modified histological silver stain was attempted in a collaborative effort by Drs. Switzer, Merrill, and Shifrin, in December, 1978, that we obtained a stain which was 100-fold more sensitive than Coomassie blue protein stains.

At least one other histological silver stain has been adapted for use in

staining proteins in polyacrylamide gels. Unfortunately, histologically-derived silver stains gave, in some cases, variable results, used large amounts of silver or other expensive reagents, or took hours to perform.

Dr. Merrill recognized that the ability of silver to form images had been used in both histology, to visualize tissue structures, and in photography, to record levels of light. In both cases, ionic silver is reduced to metallic silver. Photographic "developers" are, in general organic reducing agents. In contrast to histological stains, which have in many cases become complex, silver based photographic chemistry has become relatively simple. Image development consists of three steps: activation of silver crystals by light, selective reduction of the activated silver crystal (by a "developer"), followed by removal of non-reduced silver (in photography by a "fixer"). This photochemical procedure, when applied to a film containing silver halide crystals, results in a negative image or a photographic negative. Dr. Merrill found in May, 1980, that a negative image could also be produced in a polyacrylamide gel containing proteins. A negative image will result if a gel is soaked 10 min in silver nitrate, rinsed with water, then reduced with a photographic developer. Unreduced silver ions should be removed, just as in photography, by a photographic fixer. All of the above steps must be made in the dark. A gel image obtained in this manner contains clear regions in portions of the electrophoretogram containing protein while the rest of the gel is grayish brown. To achieve a positive image in both photography (as when using slide film) and in electrophoretic gels, "photoreversal" of the image must be accomplished. Photoreversal may be achieved by either exposure to light during image development or by chemical means. Our first positive image photoreversal silver stain used potassium ferricyanide and light to facilitate the photoreversal. Later, we found greater sensitivity could be achieved with potassium dichromate. In this manner a simplified silver staining procedure was developed.

The mechanism of silver staining is unknown. However, the fact that without photoreversal procedures a negative image is obtained, suggests that staining may be based on a Donnan type equilibrium effect. In the Donnan equilibrium, charged colloidal substances, such as proteins, alter the distribution of mobile ions, such as silver. If silver stains are based on a Donnan equilibrium, the specific effect for silver can be overwhelmed by increasing the total ionic concentration. We have found that by adding ammonium nitrate to the silver nitrate solution (to a final concentration of 1 M), the silver stain-effect is eliminated in both photochemical and histological silver staining of proteins in gels. We have also found that ammonium nitrate will block histological silver staining of brain tissue, suggesting that histological tissue staining may also be based in part on Donnan type effects.

A Donnan equilibrium effect is also consistent with the ability of silver to stain other charged polymers such as DNA and lipopolysaccharides in polyacrylamide gels. Studies on the mechanism of silver stains will be continued to facilitate further increases in sensitivity and applications.

Significance of Biological Research to the Program of the Institute:

Many diseases of the central nervous system can be diagnosed only by their symp-

toms. Evidence of genetic involvement has been obtained by family studies in some of these diseases and biochemical abnormalities have been reported in a few. The techniques developed in this project will permit surveys for trait-specific and state-specific disease protein markers on a scale that would not have been possible in the past. As an example, over 1000 proteins can be studied for alterations in molecular weight or charge in any tissue specimen. The observations of protein polymorphisms will also assist in the development of a human genetic linkage map. Such a map will be invaluable in studies of any disease with a genetic component.

Development of high sensitivity polymer (proteins, nucleic acids and polysaccharides) silver stains for the detection of such polymers following separation on gels is useful for almost all biochemical techniques in this institute. These stains permit achievements of new level of enzyme purification since trace contaminants can be visualized which could not have been seen with earlier stains. They are also essential for examination of protein patterns in tissues, particularly when the use of radioactive tracers is either prohibitively expensive or not possible (at high specific activities) as in studies of human body fluids and tissue specimens.

Proposed Course of Research:

Work on the silver stain has indicated that a further increase in sensitivity may be possible. The silver stain is currently about 200 times more sensitive than the most commonly used protein stain, Coomassie blue. With further effort, it may be possible to achieve an additional 10-fold increase in sensitivity. The use of this protein stain will permit the development of protein "maps" of brain regions, such maps will be useful for the study of pathophysiological effects and monitoring of pharmacological experiments. These methods described in this project will be used to: discover lymphocyte and fibroblast protein polymorphisms using 2DE coupled with computerized analysis; establish genetic linkage between newly discovered and previously described polymorphic loci by performing segregation analysis; search for genetic linkage between polymorphic loci and Alzheimer's and other genetic neuropsychiatric diseases; establish (with 2DE) the presence of characteristic patterns of protein modulations secondary to a primary disease process or to visualize proteins involved in the primary mutation or produced by an infectious agent responsible for the disease; and establish permanent fibroblast and lymphoblast cell lines so that studies employing other methodologies involving DNA restriction fragment polymorphisms and probes for viral sequences may be performed.

Publications:

Leavitt, J., Bushar, G., Kakunaga, T., Hamada, H., Hirakawa, T., Goldman, D., and Merrill, C.: Variations in mutant β -actin expression accompanying incremental increases in human fibroblast tumorigenicity. Cell 28: 259-268, 1982.

Leavitt, J., Goldman, D., Merrill, C., and Kakunaga, T.: Changes in gene expression accompanying chemically-induced malignant transformation of human fibroblasts. Carcinogenesis 3: 61-70, 1982.

Van Keuren, M.L., Goldman, D., and Merrill, C.R.: Protein variations associated with Down's syndrome, Chromosome 21 and Alzheimer's disease. Ann. N. Y. Acad. Sci., in press.

Goldman, D., and Merrill, C.R.: Detection of human lymphocyte polymorphisms with quantitative two-dimensional electrophoresis. Proc. Natl. Acad. Sci. U. S. A., in press.

Merrill, C.R., Goldman, D., and Van Keuren, M.L.: Simplified silver protein detection and image enhancement methods in polyacrylamide gels. Electrophoresis 3: 17-23, 1982.

Goldman, D., and Merrill, C.R.: Silver staining of DNA in polyacrylamide gels: linearity and effect of fragment size. Electrophoresis 3: 24-26, 1982.

Merrill, C.R., and Goldman, D.: Quantitative two-dimensional protein electrophoresis for studies in inborn errors of metabolism. Clin. Chem. 28: 1015-1020, 1982.

Goldman, D., Merrill, C.R., Polinsky, R.J., and Ebert, M.H.: Lymphocyte proteins in Huntington's disease: quantitative analysis by use of two-dimensional electrophoresis and computerized densitometry. Clin. Chem. 28: 1021-1025, 1982.

Leavitt, J., Goldman, D., Merrill, C., and Kakunaga, T.: Actin mutations in a human fibroblast model for carcinogenesis. Clin. Chem. 28: 850-860, 1982.

Merrill, C.R., Goldman, D., Van Keuren, M.L., and Ebert, M.H.: Molecular probes for human genetic diseases by two-dimensional protein electrophoresis and silver staining. In Electrophoresis '82, in press.

Merrill, C.R., Goldman, D., and Van Keuren, M.L.: Silver staining methods for PAGE. In Methods in Enzymology, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00942-01 LGCB |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biochemical Reactions in Mammalian Cell Chemotaxis | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: G. L. Cantoni R. R. Aksamit Other: P. S. Backlund, Jr. I.-K. Kim T. M. Caryk | Chief, Lab. Gen. Comp. Biochem. Senior Staff Fellow Staff Fellow Visiting Fellow Chemist | LGCB NIMH LGCB NIMH LGCB NIMH LGCB NIMH LGCB NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | |
| SECTION Section on Proteins | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 1.5 | OTHER: 1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Chemotaxis by a macrophage cell line was inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin. Determination of the change in intracellular levels of S-adenosyl-L-homocysteine (AdoHcy) and 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) showed that inhibition of chemotaxis was correlated with the increase in 3-deaza-AdoHcy, formed intracellularly by the utilization of 3-deazaadenosine as a substrate for AdoHcy hydrolase. Two lipid reactions were tested for susceptibility to inhibition by 3-deazaadenosine and 3-deazaaristeromycin. Both the synthesis of phosphatidylcholine by stepwise methylation of phosphatidylethanolamine and the release of arachidonic acid were inhibited by 3-deazaadenosine and 3-deazaaristeromycin, indicating that these two reactions were not required for chemotaxis. However, the synthesis of a small number of proteins, separated by two-dimensional polyacrylamide gel electrophoresis was susceptible to 3-deazaadenosine, but not to 3-deazaaristeromycin. A correlation was found between inhibition of chemotaxis and inhibition of the synthesis of the same subset of proteins when other compounds were tested. We postulate that incubation of cells with 3-deazaadenosine inhibits a methylation reaction that is required for the formation of a functional mRNA coding for one or more proteins required for chemotaxis. | | |

Project Description:

The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy has allowed us to assess the significance of certain biochemical reactions in macrophage chemotaxis. For these studies we chose the cloned RAW264 mouse macrophage cell line which is easily grown in tissue culture and is amenable to genetic manipulation. Other studies of leukocyte chemotaxis have had the disadvantage that the cells were heterogeneous with respect to cell type and cellular behavior. Chemotaxis by RAW264 cells is inhibited by 3-deaza-AdoHcy, formed by AdoHcy hydrolase when 3-deazaadenosine is administered to the cells. In addition to the accumulation of 3-deaza-AdoHcy, AdoHcy also accumulates due to inhibition of AdoHcy hydrolase. Fortunately, another inhibitor of AdoHcy hydrolase, 3-deazaaristeromycin, has been developed in this laboratory that inhibits AdoHcy hydrolase but does not function as a substrate for the enzyme. Administration of 3-deazaaristeromycin to the cells resulted in the accumulation of AdoHcy to levels that are higher than those achieved by administration of 3-deazaadenosine. Therefore, since 3-deazaaristeromycin had no effect on chemotaxis, it was possible to conclude that chemotaxis was specifically inhibited by 3-deaza-AdoHcy.

A search was initiated for a reaction that was sensitive to 3-deaza-AdoHcy. Neither the synthesis of phosphatidylcholine by methylation of phosphatidylethanolamine nor the release of arachidonic acid when the cells were incubated with EAMS (endotoxin-activated mouse serum, an attractant for mouse macrophages) was specifically sensitive to 3-deazaadenosine. From these studies we conclude that, unlike the results reported from other laboratories, neither the synthesis of phosphatidylcholine from phosphatidylethanolamine nor the release of arachidonic acid are required reactions for chemotaxis by RAW264 cells.

A reaction was found that was inhibited when cells were incubated with 3-deazaadenosine but not with 3-deazaaristeromycin. The reaction is the inhibition of the synthesis of one or a small number of proteins, separated by two-dimensional polyacrylamide gel electrophoresis. The possible relationship of this reaction to chemotaxis was strengthened by the finding that other inhibitors of chemotaxis inhibited the synthesis of the same subset of proteins. These inhibitors are 3'-deoxyadenosine and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) in the presence of adenosine and homocysteine. A common feature of the inhibitors of chemotaxis described above is that all of them can inhibit the synthesis of functional mRNA. In this regard, we have also found that inhibitors of protein synthesis and translation, such as cycloheximide, puromycin, and actinomycin D, inhibit chemotaxis.

Significance of Biological Research to the Program of the Institute:

AdoMet-dependent transmethylation reactions are involved in the synthesis of DNA, RNA, proteins, lipids and neurotransmitters. Inhibitors of transmethylation have been shown to affect cell differentiation, virus replication and mammalian cell chemotaxis. Mammalian cell chemotaxis is one of the most important reactions in the immune response and development of the central nervous system.

For the elucidation of the biochemical reactions of the mammalian cell chemotaxis,

we have studied and found interesting and useful results: (a) chemotaxis was specifically inhibited by 3-deaza-AdoHcy; (b) phospholipid methylation has no relation to chemotaxis; and (c) one or a smaller number of proteins are involved in the chemotaxis. However, the specific biochemical reactions involved in mammalian cell chemotaxis are not clear and need more study, especially chemotaxis-related specific proteins and details of the molecular levels.

We will be able to explain the relationships between the transmethylation, chemotactic reaction, the immune response and the development of the central nervous system.

Proposed Course of Research:

Further work will be directed toward verification of the hypothesis that 3-deaza-AdoHcy specifically inhibits the synthesis of functional mRNA coding for one or more proteins required for chemotaxis, and toward the identification of biochemical reactions important in chemotaxis. Chemotaxis is a reaction important not only to the immune response, but also the development to the central nervous system.

Publications:

Aksamit, R.R., Falk, W., and Cantoni, G.L.: Inhibition of chemotaxis by S-3-deazaadenosylhomocysteine in a mouse macrophage cell line. J. Biol. Chem. 257: 621-625, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00943-01 LGCB |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Pathways of Methionine and Threonine Metabolism, and Their Control, in Higher Plants | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | J. Giovannelli K. Veluthambi S. H. Mudd A. H. Datko G. A. Thompson | Research Chemist Visiting Fellow Chief, Section on Alkaloid Biosynthesis Botanist Senior Staff Fellow LGCB NIMH LGCB NIMH LGCB NIMH LGCB NIMH LGCB NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of General and Comparative Biochemistry | | |
| SECTION Section on Alkaloid Biosynthesis | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2-3/12 | PROFESSIONAL: 2-1/2 | OTHER: 2/12 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Preliminary estimates of the physiological rates of <u>methylneogenesis</u> , <u>trans-methylation</u> , and <u>methionine thiomethyl recycling</u> in <u>Lemna</u> indicate that these processes are not regulated by <u>methionine</u> or one of its products. The following evidence has been obtained to support our previous proposal that methionine thio-methyl recycling proceeds via conversion of <u>methylthioadenosine</u> to methionine: (i) The growth of <u>Lemna</u> deprived of methionine by administration of lysine + threonine and/or propargylglycine is restored by supplementation with methylthioadenosine. (ii) Incubation of <u>Lemna</u> with methylthioadenosine labeled in the methyl and adenosyl moieties indicate that the methylthio and 4-carbon moieties of methionine were derived, respectively, from the methylthio and four ribose carbons, of methylthioadenosine. (iii) Cell-free extracts of <u>Lemna</u> convert methylthioadenosine to methionine, with <u>methylthioribose-1-phosphate</u> tentatively identified as an intermediate. A sensitive and specific assay was developed for <u>threonine synthase</u> , and optimal conditions established for its assay, extraction and stability. The enzyme from <u>Lemna</u> resembles that described from a variety of plants by other workers in being markedly stimulated by <u>S-adenosylmethionine</u> . | | |

Project Description:

The major findings of our work on methionine metabolism are as follows:

(1) We have previously shown that the individual parts of the methionine molecule (the methyl group, sulfur atom and 4-carbon portion) are synthesized de novo at different rates. It is clear that a comprehensive understanding of the overall process of regulation of methionine biosynthesis can be obtained only from an understanding of how the synthesis of each individual part of methionine is regulated, and how these individual regulatory processes are integrated. As an initial step in solving this complicated problem, we have estimated the rates of methylneogenesis, transmethylation, and methionine thiomethyl recycling in Lemna growing in the presence of only tracer concentrations of double-labeled methionine that would not significantly increase the pool sizes of soluble methionine and S-adenosylmethionine. Within the limits of precision of our analyses, these estimates were not different to those previously determined with Lemna growing in concentrations of methionine that increased the pool sizes of S-adenosylmethionine and soluble methionine by one and two orders of magnitude, respectively. These results suggest that methionine (or one of its products such as S-adenosylmethionine) does not regulate either methylneogenesis, transmethylation or methionine thiomethyl recycling under conditions that we have shown decrease methionine synthesis via transsulfuration by 70 to 80%.

(2) We now have convincing evidence for conversion of methylthioadenosine to methionine by a process in which methylthioadenosine provides both the thiomethyl and 4-carbon moieties of methionine. This evidence is as follows:

(i) The growth of plants that are deprived of methionine by administration of lysine + threonine or propargylglycine (either separately or in combination) can be restored by supplementation with methylthioadenosine. This and other supporting data show that methylthioadenosine can provide the amounts of methionine that are needed for normal growth. Lysine + threonine inhibits in vivo synthesis of homoserine, and hence of O-phosphohomoserine; propargylglycine is a potent suicide inhibitor of cystathionine γ -synthase. The ability of methylthioadenosine to provide methionine when either or both O-phosphohomoserine synthesis and cystathionine γ -synthase are inhibited is entirely consistent with methylthioadenosine providing both the thiomethyl and 4-carbon moieties of methionine, and argues against alternative schemes in which O-phosphohomoserine provides the 4-carbon moiety of methionine by a reaction requiring cystathionine synthase.

(ii) Incubation of Lemna with methylthioadenosine labeled with ^3H (in the methyl group) and ^{14}C (in the adenosyl moiety) demonstrated an efficient conversion of carbon atoms from both moieties into methionine and its products. Four adenosyl carbons for each methyl carbon of methylthioadenosine were incorporated into methionine. This stoichiometry, together with the characteristic labeling patterns formed from the two labeled moieties of methylthioadenosine, strongly support a scheme in which the methylthio and 4-carbon moieties of methionine are derived, respectively, from the methylthio and four ribose carbons of methylthioadenosine.

(iii) Cell-free extracts of Lemna convert methylthioadenosine to methionine. While Lemna utilized methylthioribose for methionine synthesis in vivo, cell-free

extracts did not catalyze this conversion under conditions in which methylthioadenosine was used. These data suggest that methylthioribose may not be a normal intermediate in methionine synthesis from methylthioadenosine. In support of this suggestion, methylthioribose-1-phosphate was found as a product of methylthioadenosine metabolism. Although work with cell-free extracts is still in a preliminary stage, these combined results are consistent with methylthioadenosine being cleaved initially by phosphorolysis to adenine and methylthioribose-1-phosphate, which is then further metabolized to methionine and a one-carbon fragment.

The demonstration that methylthioadenosine provides both the methylthio and 4-carbon moieties of methionine contributes to our understanding of the pathway of methionine thiomethyl recycling. In addition, this finding allows us to interpret previous experiments on the incorporation of sulfate ³⁵S into cystathionine and its products as proving that supplementary methionine caused a 70-80% inhibition of flux through cystathionine γ -synthase.

The following progress has been made during the short time devoted to our studies on threonine biosynthesis:

- (1) A sensitive and specific assay for threonine synthase, which catalyzes the synthesis of threonine from O-phosphohomoserine, was developed.
- (2) Optimal conditions for assay, extraction and stability of threonine synthase from Lemna were established. The enzyme from Lemna resembles that described from a variety of plants by other workers in being markedly stimulated by S-adenosylmethionine. At limiting concentrations of O-phosphohomoserine, S-adenosylmethionine stimulated threonine synthesis over 30-fold; stimulations were progressively less as O-phosphohomoserine concentration approached saturation. A variety of extraction, assay and storage conditions did not significantly affect the stimulation by S-adenosylmethionine.
- (3) Propargylglycine is a potent suicide inhibitor of a variety of pyridoxal phosphate enzymes (including cystathionine γ -synthase) that require labilizing of the β -hydrogen on the substrate. While threonine synthase appears to conform to this requirement, no significant inhibition by propargylglycine (either in the absence or presence of S-adenosylmethionine) was observed under conditions that caused essentially complete inhibition of Lemna cystathionine γ -synthase.

Significance of Biomedical Research to the Program of the Institute:

Efforts continue to focus on our primary goal of elucidating the pathways of methionine biosynthesis and metabolism, and their control, in higher plants, using Lemna as the experimental system. This year we have also initiated parallel studies with threonine, another essential amino acid of the aspartate family. Methionine and threonine biosynthesis are closely related in plants, the two pathways branching at the common intermediate O-phosphohomoserine. There are now a number of indications that regulation of the two biosynthetic branches may also be interrelated. We expect that the recently-initiated studies with threonine will complement analogous studies with methionine, and ultimately yield a comprehensive picture of how the synthesis of these essential amino acids is regulated.

This project is significant to the research goals of the Institute since methionine and threonine are among the four most commonly limiting essential amino acids in the human diet. Deficiency of these amino acids (especially during early life) in protein-calorie malnutrition may be accompanied by irreversible retardation in mental development. Plant proteins provide the source of almost all these two amino acids, either directly by ingestion of plant material, or indirectly through an animal intermediate. Many of the plant foods most used by man are deficient in one or both of the amino acids, methionine and threonine. An understanding of the patterns of control of the biosynthesis and metabolism of methionine and threonine will provide a rational basis for maximizing the production of these essential dietary components.

Proposed Course of Research:

- (1) The experiments performed with double-labeled methionine have provided the first quantitative estimates of the relative amounts of methionine metabolized in vivo through the major pathways, such as incorporation into protein, polyamine synthesis and transmethylation. The various pathways through which methionine are metabolized will be defined in more detail by use of methionine labeled specifically in the appropriate moiety, and the quantitative fluxes through these pathways will be determined.
- (2) Studies on the cell-free synthesis of methionine from methylthioadenosine will be continued in an effort to optimize the system, and to define the intermediates in the pathway. Of special interest is whether the pathway in plants proceeds via methylthioribose (as in bacteria) or methylthioribose-1-phosphate (as in animals).
- (3) While it is suggested that methionine thiomethyl recycling is probably important in conserving the thiomethyl (and adenine) moieties of methylthioadenosine and in preventing the build-up of inhibitory concentrations of methylthioadenosine formed from various reactions of S-adenosylmethionine metabolism (such as polyamine synthesis), the physiological importance of the recycling has not been clearly defined. We are currently attempting to find inhibitors of the conversion of methylthioadenosine to methionine that should help assess the physiological significance of methionine thiomethyl recycling. Such inhibitors might also be expected to help in clarifying the intermediates in this pathway.
- (4) By a variety of methods, including the use of methionine specifically labeled in the appropriate moiety, we plan to examine further how transmethylation (and recycling of the homocysteine moiety of methionine) and polyamine synthesis (and methionine thiomethyl recycling) are regulated, and how these regulatory processes are integrated.
- (5) Threonine synthase, which has not yet been extensively purified from plants, will be purified from Lemna, and its catalytic and regulatory properties will be examined. Special attention will be paid to the interesting allosteric stimulation by S-adenosylmethionine.
- (6) Lemna will be grown under conditions of threonine deficiency or surplus, and the activities of threonine synthase will be assayed to determine whether it

is subject to non-allosteric alterations. The physiological significance of any such alteration in the control of threonine (and methionine) synthesis will be assessed.

(7) Labeling experiments will be performed with intact Lemna to determine the extent of regulation of threonine synthesis that occurs in vivo, and the compounds that exert this regulation.

Publications:

Macnicol, P.K., Datko, A.H., Giovanelli, J., and Mudd, S.H.: Homocysteine biosynthesis in green plants: Physiological importance of the transsulfuration pathway in Lemna paucicostata. Plant Physiol. 68: 619-625, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00981-17 LNB |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Analysis of the Macromolecular Structure of Nerve Membrane during Excitation | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: OTHERS: | Ichiji Tasaki Kunihiko Iwasa Paul M. Byrne Toshiro Inubushi | Chief Sr. Staff Fellow Biomed. Engr. Tech. Visiting Fellow LNB NIMH LNB NIMH LNB NIMH LCP NIADDK |
| COOPERATING UNITS (if any) Laboratory of Chemical Physics, NIADDK and Marine Biological Laboratory, Woods Hole, MA | | |
| LAB/BRANCH Laboratory of Neurobiology SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 2.7 | PROFESSIONAL: 2 | OTHER: 0.7 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> We have continued and expanded our analysis of the phenomenon of <u>swelling of the nerve fiber during excitation</u>. (This phenomenon was discovered in this Laboratory about two years ago.) After establishing the fact that the degree of swelling reaches its maximal level at the peak of the internally recorded action potential in the <u>squid axon</u>, the following four types of investigation were carried out: (1) determination of the time-course of rapid <u>mechanical responses</u> of the squid axon treated with tetraethylammonium salt, (2) analysis of nerve swelling during potassium- and veratridine-depolarization, (3) measurements of nerve swelling associated with repetitive excitation, and (4) studies of ion and water movements across the nerve membrane during potassium depolarization. The importance of water movements during excitation was amply demonstrated by these investigations. Since the time-resolution of our mechanical measurements is very high, we expect to gain a further insight into the process of nerve excitation by accurately determining the time-courses of mechanical changes in the nerve fiber under a variety of experimental conditions. </p> | | |

Project Description:

Objectives:

According to the theory of nerve excitation proposed originally by Jacques Loeb and recently extended by the principal investigator (see ref. 1), transitions of membrane macromolecules between a compact and a swollen state constitute a cycle of excitation and recovery. Our recent finding that the nerve membrane swells in phase with the depolarization phase of the action potential is quite consistent with the theory. The objective of the present research is to critically examine whether or not closer analyses of mechanical changes in the nerve fiber lends further support to this theory.

Methods Employed:

(1) A sensitive pressure-detector was devised by using a thin film of polyvinylidene fluoride as the piezoelectric element. The signal-to-noise ratio in detection of small pressure changes in the nerve fiber with this new device was found to be about 3 times better than that in our previous measurements using a ceramic piezoelectric bender as a detector. By using this new device, we could determine the time-course of pressure changes in squid giant axons treated internally with tetraethylammonium (TEA) salt. (2) Weight changes of the nerve fibers were determined by weighing the intact nerve before and after treatment. (3) Mechanical changes in the nerve associated with repetitive stimulation were measured using an extremely small lever in conjunction with a Fotonic sensor. (4) The process of uptake of water, cations and anions by the nerve fiber was studied by using radio-isotopes, as well as by means of proton nuclear-magnetic resonance spectroscopy (NMR).

Major Findings:

(1) Associated with a prolonged action potential of a TEA-treated axon, minute swelling of the axon was observed only at the onset of the action potential. During the plateau of the action potential, there was a progressively increasing shrinkage of the axon. Under these conditions, there was a pronounced disparity in the time-course between the action potential and the mechanical change. The observed shrinkage of the axon during the plateau may be regarded as a reflection of a relatively low membrane conductance. The mechanical changes were very sensitive to variations in the external Ca^{2+} concentration.

(2) We found that, during potassium- and veratridine-depolarization, the weight of a crab nerve (trunk) gradually increases by taking up water from the medium. The degree of swelling observed under these conditions was found to depend strongly on the anion species in the medium. Anions with greater lyotropic numbers (e.g., iodide or thiocyanide) giving rise to a greater degree of swelling. The difference in the degree of swelling is considered to reflect a variation among anions in their ability to solubilize cytoskeletal proteins.

(3) The time-course of shortening of the nerve fiber during repetitive stimulation at a high frequency was found to produce a sustained contraction which resembles tetanus in vertebrate skeletal muscles. A significant difference in mechanical behavior between the crab nerve and squid giant axon was recognized.

(4) We found that in crab nerve fibers and squid giant axons immersed in a potassium-rich medium, there is an enhanced uptake of various anions; uptake of chaotropic anions is accompanied by an increase in the water-content of the fibers. We have shown that Mn^{2+} ions --- paramagnetic ions capable of suppressing NMR signals of the water molecules in the vicinity --- are taken up by depolarized nerve fibers. (Note that Mn ions had been used for the purpose of distinguishing intracellular H_2O from extracellular H_2O by erroneously assuming impermeability of the nerve membrane to these transition metal ions.) Currently, we are planning to expand the use of NMR spectroscopy in our further study of water movements in the nerve fiber.

Scientific Significance and Relevance to Public Mental Health:

The scientific significance of this work is that it provides a critical examination and analysis of a variety of mechanical changes associated with nerve excitation. The significance of these changes (swelling, shrinkage and shortening of the nerve fiber associated with excitation) is that they lend further support to the macromolecular theory of nerve excitation (see ref. 1).

Proposed Course:

Currently, we are planning to expand the use of NMR spectroscopy and other physical measurements in our further studies of water movements in the nerve fiber. In addition, we plan to continue to expand our studies of the role of cytoskeletal proteins in maintaining both excitability and the morphology of nerve fibers.

Publications:

Tasaki, I.: Physiology and Electrochemistry of Nerve Fibers. Vol. 3, A. Noordergraaf (Ed.): Academic Press, New York, 1982, 348 pp.

Tasaki, I., and Terakawa, S.: Oscillatory miniature responses in the squid giant axon: Origin of rhythmical activities in the nerve membrane. In Carpenter, D. (Ed.): Cellular Pacemakers. John Wiley, 1982, Vol. 1, pp. 163-186.

Tasaki, I., and Iwasa, K.: A Physicochemical Approach to Calcium Membrane-macromolecule Interactions. In Ohnishi, S.T. and Endo, M. (Eds.): Mechanism of Gated Calcium Transport Across Biological Membranes. Academic Press, 1982, pp. 183-189.

Tasaki, I., and Iwasa, K.: Rapid mechanical changes in crab nerve and squid axon during action potentials. J. Physiol. (Paris), in press.

Iwasa, K., and Tasaki, I.: Swelling and Shrinkage of Nerve Fibers Associated with Action Potentials. In Martonosi, A. (Ed.): Membrane and Transport. Plenum, 1982, pp. 385-388.

Tasaki, I., and Iwasa, K.: Temperature changes associated with nerve excitation: Detection by using polyvinylidene fluoroide film. Biochem. Biophys. Res. Comm. 101: 172-176, 1981.

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- Iwasa, K.: An application of the osmotic equation to a polyelectrolyte solution. J. Chem. Phys. 74: 5848-5850, 1981.
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- Iwasa, K., and Tasaki, I.: Excitable membrane and cytoskeleton: Axolemma-ectoplasm complex. Seitai no Kagaku 32: 30-35, 1981.
- Metuzals, J., Tasaki, I., Terakawa, S., and Clapin, D. F.: Removal of the Schwann sheath from the giant nerve fiber of the squid: An electron-microscopic study of the axolemma and associated axoplasmic structures. Cell Tissue Res. 221: 1-15, 1981.
- Tasaki, I., and Byrne, P. M.: Tetanic contraction of the crab nerve evoked by repetitive stimulation. Biochem. Biophys. Res. Commun., in press.
- Terakawa, S.: Ca-K bi-ionic action potential in squid giant axon. J. Membrane Biol. 63: 41-50, 1981.
- Terakawa, S.: Periodic response in squid axon membrane exposed intracellularly and extracellularly to solutions containing a single species of salt. J. Membrane Biol. 63: 51-59, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00983-04 LNB |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biochemical Studies on the Mechanism of Nerve Excitation | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: OTHERS: | Jesse Baumgold Paul Gallant Irma Zimmerman Ilan Spector J. Brian Parent | Sr. Staff Fellow Staff Fellow Biological Tech. Scientific Expert Research Associate LNB NIMH LNB NIMH LBG NHLBI Howard Univ. Cancer Center |
| COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA | | |
| LAB/BRANCH Laboratory of Neurobiology | | |
| SECTION NIMH, ADAMHA, NIH, Bethesda, MD 20205 | | |
| INSTITUTE AND LOCATION | | |
| TOTAL MANYEARS: 3.2 | PROFESSIONAL: 2.2 | OTHER: 1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The <u>development of excitability</u> was studied in <u>nerve and muscle cells in culture</u> by measuring <u>neurotoxin binding</u> , <u>²²Na-uptake</u> and <u>electrophysiological parameters</u> of the cells. In embryonic muscle and nerve cells in the normal culture medium, <u>³H-saxitoxin binding</u> , batrachotoxin-stimulated <u>²²Na-uptake</u> and electrical excitability appeared gradually, reaching maximal values by about 8 days in culture. In the presence of scorpion toxin in the medium, <u>¹²⁵I-scorpion toxin binding</u> , batrachotoxin + scorpion toxin stimulated <u>²²Na-uptake</u> and excitability appeared sooner and reached maximal values by about 4 days in culture. These data suggest that excitable site proteins are incorporated into the cell membrane in an inactive form and they undergo a <u>post-translational modification</u> which render them active. A monoclonal antibody is currently being used to purify the excitable site protein. Using <u>squid giant axons</u> , the biochemical processes underlying the deteriorative action of a rise in the internal Ca-ion concentration on excitable membrane sites was examined. It is shown that activation of an endogenous <u>protease</u> by Ca-ion is one of the major factors responsible for suppression of excitability. | | |

START

Project Description:

Objectives:

The transmission of electrical impulses along a nerve or muscle fiber is mediated by specialized proteins in the membrane known as excitable sites or channels. The electrophysiological properties of these sites or channels have been extensively studied, but the molecular characteristics of these specialized proteins have yet to be elucidated. It is the general goal of this research project to elucidate the nature of the proteins which render nerve and muscle cells excitable. One of these proteins, frequently referred to as a sodium channel, is known to have three distinct neurotoxin binding sites. Since the electrophysiological effect of each of these three neurotoxins is quite different, one specific goal of this project is to determine whether these three neurotoxin binding sites are associated with the same or with different subunits of the sodium channel.

During the course of cellular differentiation, myoblasts and neuroblasts, the undifferentiated and electrically inexcitable precursors for muscle and nerve cells, gradually acquire the ability to develop action potentials. Since these myoblasts and neuroblasts can be grown and induced to differentiate in culture, they provide a very convenient system for studying the development of excitability.

The specific goals of this project are to: 1) use this developmental system to correlate the appearance of excitability with the appearance of each of the neurotoxin binding sites 2) study the process by which nerve and muscle cells become excitable and how this process is biologically regulated.

It has been hypothesized that the major factor leading to Wallerian degeneration of nerve fibers is the influx of Ca-ion through the nerve membrane under abnormal conditions. In another series of experiments, we have sought to elucidate the role played by intracellular Ca in the process of suppression of excitability in the squid giant axon.

Methods Employed:

1) The appearance of certain proteins known to be involved in nerve excitation was followed during development by using two different radioactively labeled neurotoxins. These neurotoxins (^3H -saxitoxin and ^{125}I -scorpion toxin), which were purified and isolated in this laboratory, bind specifically to different areas of excitable sites (sodium channels). 2) The sensitivity of the excitable sites to various drugs and neurotoxins was followed, during the course of development, by studying the ability of these cells in culture to take up ^{22}Na from the medium in response to various drugs and toxins. 3) The cells were impaled with microelectrodes and the electrophysiological responses of the cells could thus be assessed at various stages of differentiation. 4) The role of intracellular Ca-ion in suppressing excitability was studied by electrophysiological recording techniques and the proteolysis induced by Ca-ion was monitored by polyacrylamide gel electrophoresis as well as an assay involving C-14 casein.

START

Major Findings:

The following results were obtained by the methods described above. In all three sets of measurements, two different developmental schedules were found: a relatively slow development was found in cells cultured in the normal medium, whereas a much faster developmental schedule was found in cells treated with scorpion toxin. Specifically, the binding studies with the neurotoxins revealed that in the absence of scorpion toxin, ^3H -saxitoxin binding sites developed gradually and reached a plateau value by 8 days in culture. In contrast, when the excitable sites were assayed using ^{125}I -scorpion toxin, the binding sites for this toxin developed rapidly and reached a plateau by 4 days in culture. Analogous results were found with the ^{22}Na -uptake studies: in the absence of saxitoxin (ScTX), the batrachotoxin-stimulated ^{22}Na -uptake reached a plateau after 8 days in culture, whereas in the presence of scorpion toxin, it reached a plateau after 4 days in culture. Finally, the electrophysiology showed that 4 day old cultures were inexcitable in the absence of ScTX but that the addition of ScTX rendered these cells excitable within minutes.

Based on these and other data, we developed a comprehensive hypothesis for the development of excitable sites (sodium channels). This hypothesis states that the protein which forms these excitable sites, are incorporated into the cell membrane initially in an inactive form and, during the process of cellular differentiation, undergoes a post-translational modification which renders them active. Treatment of the cells with scorpion toxin is hypothesized to greatly speed up this post-translational modification so that, in the presence of this toxin, the immature cells become electrophysiologically mature in a matter of minutes rather than the days required for this to occur in the absence of the toxin.

It was found when the calcium ion concentration within a squid axon was raised to millimolar levels, calcium dependent proteolysis of cytoskeletal proteins occurred and excitability was suppressed. These two actions of calcium, proteolysis and suppression of excitability, could be dissociated in dialysed axons by exposing the axon to leupeptin. Leupeptin inhibits proteolysis without affecting the calcium induced loss of excitability. Another way of dissociating these two actions of calcium is by poisoning the axon with cyanide. Under this condition, the intracellular calcium concentration becomes elevated, leading to loss of excitability after 5 to 10 hours. Proteolysis, however, was not detected until after 15 hours. These results suggest that Ca-dependent proteolysis is not the only effect of raising intracellular calcium levels. It is expected that studies along this line be completed by the end of the present fiscal year.

Scientific Significance and Relevance to Public Mental Health:

Although extremely detailed descriptions of the electrical events occurring during nerve and muscle excitation have been described in the literature, virtually nothing is known about the proteins involved in these processes. The work described in this project is aimed at elucidating, for the first time, the nature of the proteins involved in nerve and muscle excitation. The significance of this work, thus lies in the fact that it is a first attempt at understanding the biochemistry underlying the process of nerve and muscle excitation, processes that are clearly vital to the survival of man.

Proposed Course:

We are currently in the process of testing our hypothesis with the following experiments. Our hypothesis predicts the existence of two slightly different excitable sites (sodium channels): an immature, non-functional one found in immature cells and a mature, functional one in mature, differentiated cells. We therefore are planning to isolate and purify these proteins from both mature and immature cells in the hopes of being able to demonstrate these differences. We have recently obtained a monoclonal antibody against this protein. We plan to use this antibody to make an affinity column which would greatly facilitate this purification.

Since, in the past year, we have developed a number of very powerful tools with which to study the biochemistry of excitability (including radio-labeled neurotoxins such as ^3H -saxitoxin, ^{125}I -scorpion toxin, unlabeled neurotoxins that can be used in ^{22}Na -uptake studies and a monoclonal antibody), we are also planning to use these tools to study the biochemistry of excitability in a number of diseases for which animal models are available. Specifically, we plan to study whether excitable sites (especially sodium channels) are affected by diseases such as muscular dystrophy and in experimental allergic encephalitis, an animal model for multiple sclerosis. Since it is not yet known whether excitable sites (sodium channels) are involved in diseases that affect nerve conduction, it is still too early to know what clinical relevance our work will have. However, by addressing ourselves to these questions, our work becomes very clinically relevant.

Publications:

Baumgold, J., Gallant, P., Terakawa, S., and Pant, H.: Tetrodotoxin affects submembranous cytoskeletal proteins in perfused squid giant axons. Biochem. Biophys. Res. Comm. 103: 653-658, 1981.

Fink, D. J., Russell, J. T., Gainer, H., Brownstein, M. J., Baumgold, J.: Multiple-rate components of axonally transported proteins in the hypothalamo-neurohypophyseal system of the rat. J. Neurobiol. 12: 487-503, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 00985-01 LNB * | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 * | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Regulation of Protein and Enzyme Function by Modulator-Sites on Complex Carbohydrates | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;"> PI: </td> <td style="width: 35%; vertical-align: top;"> Audrey L. Stone </td> <td style="width: 35%; vertical-align: top;"> Research Chemist </td> <td style="width: 15%; vertical-align: top;"> LNB NIMH </td> </tr> <tr> <td style="vertical-align: top;"> OTHERS: </td> <td style="vertical-align: top;"> Robert D. Rosenberg </td> <td style="vertical-align: top;"> Prof. of Med. & Prof. of Biochem. </td> <td style="vertical-align: top;"> Harvard Med. Sch., Mass. Inst. Technol. </td> </tr> <tr> <td></td> <td style="vertical-align: top;"> Gary M. Oosta </td> <td style="vertical-align: top;"> Research Associate </td> <td style="vertical-align: top;"> Harvard Med. Sch. </td> </tr> <tr> <td></td> <td style="vertical-align: top;"> David Beeler </td> <td style="vertical-align: top;"> Research Technician </td> <td style="vertical-align: top;"> Harvard Med. Sch. </td> </tr> <tr> <td></td> <td style="vertical-align: top;"> Elizabeth Walker </td> <td style="vertical-align: top;"> Chemist </td> <td style="vertical-align: top;"> Mass. Gen. Hospital </td> </tr> </table> | | | PI: | Audrey L. Stone | Research Chemist | LNB NIMH | OTHERS: | Robert D. Rosenberg | Prof. of Med. & Prof. of Biochem. | Harvard Med. Sch., Mass. Inst. Technol. | | Gary M. Oosta | Research Associate | Harvard Med. Sch. | | David Beeler | Research Technician | Harvard Med. Sch. | | Elizabeth Walker | Chemist | Mass. Gen. Hospital |
| PI: | Audrey L. Stone | Research Chemist | LNB NIMH | | | | | | | | | | | | | | | | | | | |
| OTHERS: | Robert D. Rosenberg | Prof. of Med. & Prof. of Biochem. | Harvard Med. Sch., Mass. Inst. Technol. | | | | | | | | | | | | | | | | | | | |
| | Gary M. Oosta | Research Associate | Harvard Med. Sch. | | | | | | | | | | | | | | | | | | | |
| | David Beeler | Research Technician | Harvard Med. Sch. | | | | | | | | | | | | | | | | | | | |
| | Elizabeth Walker | Chemist | Mass. Gen. Hospital | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Harvard Medical School, Massachusetts Institute of Technology | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neurobiology | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; border-bottom: 1px solid black;">TOTAL MANYEARS: 1.4</td> <td style="width: 33%; border-bottom: 1px solid black;">PROFESSIONAL: 1.2</td> <td style="width: 33%; border-bottom: 1px solid black;">OTHER: 0.2</td> </tr> </table> | | | TOTAL MANYEARS: 1.4 | PROFESSIONAL: 1.2 | OTHER: 0.2 | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.4 | PROFESSIONAL: 1.2 | OTHER: 0.2 | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Heparin-like complex carbohydrates</u> activate the esterase inhibitor, <u>antithrombin</u> , and <u>tyrosine hydroxylase</u> from the <u>CNS</u> in a manner that involves multiple modulating interactions. Heparin promotes two activated states in antithrombin: Its major binding site (unique sequence) activates against factor X_2 but not thrombin, while binding of this site plus a secondary region (about eight saccharides away) activates the inhibitor against both enzymes. Structure-function relations of newly isolated <u>heparin octa-to-octadecasaccharides</u> along with <u>model sugar units</u> are investigated using <u>intrinsic</u> and <u>extrinsic circular dichroism spectroscopy</u> . A disaccharide sequence is proposed which is the first to elucidate the structure of the second binding region. Conformational basis for multiple regulatory movements in antithrombin are explored by fluorescence and circular dichroism spectroscopy of antithrombin complexes with <u>octa-to-octadecasaccharides</u> and higher molecular weight fractions and by kinetic analysis. These studies provide the first evidence for a conformational difference between the two activated states of antithrombin. Molecular basis of activation of tyrosin hydroxylase by heparin is investigated by <u>in vitro</u> kinetic analysis using partially purified enzyme plus heparin fractions <u>in combination with newly identified peptide inhibitors</u> . | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: 1) To determine the molecular basis for heparin-like modulation of enzyme systems and protein regulators; to develop models for protein-polysaccharide interactions and for directed protein-protein approximation which mimic developmental processes such as cell-cell recognition and directed neuronal cell elongation; to isolate heparin oligosaccharides and human antithrombin, form their complexes and measure protein conformational aspects with advanced circular dichroism techniques. 2) To characterize and determine the structural features of heparin modulating oligo- and polysaccharides that relate to their action on enzyme systems including those in the CNS. To elucidate the nature and sequence of saccharides of the second binding region of heparin. To elucidate polysaccharide features (conformational/structural) of other special amino-sugar containing glycosaminoglycans. 3) To initiate new studies into the possible role of heparan sulfates, as modulators of enzymatic and/or developmental functions in the CNS: To characterize oligo- and polysaccharide heparan sulfate fractions from patients with Sanfilippo mucopolysaccharidoses; to identify their possible role in normal and pathological development especially as it relates to the mental retardation associated with these disorders.

Methods Employed: Standard chromatographic techniques were used to isolate the coagulation esterases, antithrombin, heparin fractions of 22 Kd and 6500d and other complex carbohydrates. Affinity-gel techniques were generated with Con A- and heparin-sepharose for isolation of antithrombin-bound heparins and tyrosine hydroxylase, respectively. Oligosaccharides were generated by controlled nitrous acid cleavage. Assays of biological activity of the coagulation enzymes and heparins were spectrophotometric and developed in Dr. Rosenberg's laboratory, while that for tyrosine hydroxylase was a radioassay developed at NIH using sub-optimal cofactor levels. Physical characterizations employed the Cary models 60 and 61 and the Jasco 500J spectropolarimeters under newly specified conditions and the Perkin-Elmer spectrofluorimeter. Concentration of complex carbohydrates was determined by the carbazole assay for uronic acids. Standardized metachromatic ligands were used to titrate the number and the type of charge distributions as well as conformational aspects of the bound ligand and its binding site.

Major findings: 1) Two types of chiro optical effects emerge upon formation of the biologically active complexes that correlate with the two activated states of antithrombin. The first spectral pattern is observed when octasaccharide, decasaccharide, dodecasaccharide or tetradecasaccharide fragments bind to the protease inhibitor and indicates perturbation of "buried" and "exposed" tryptophan residue(s). Given that these heparin fragments are only able to accelerate factor Xa-antithrombin but not thrombin-antithrombin interactions, the spectral transitions must be associated with the binding of oligosaccharides to the primary recognition site of the protease inhibitor and/or the "activation" of the protease inhibitor with respect to factor Xa neutralization.

The second pattern is noted when octadecasaccharide, LMW (~6,500 d) or HMW (~22,000 d) heparin bind to antithrombin. These complexes exhibit CD difference spectra that are similar to that of the first pattern except for changes (within the 296 nm - 282 nm and 270 nm - 255 nm regions) that are consistent with perturbations of a disulfide bridge with or without contributions

arising from effects on an additional tryptophan. Given that the three mucopolysaccharides are able to accelerate both thrombin-antithrombin and factor Xa-antithrombin interactions, the difference between the two above chiral patterns should correspond to heparin-induced "activation" of the protease inhibitor with respect to thrombin neutralization. Thus, the two major regions of anticoagulant active heparin probably interact with separate areas of antithrombin and appear to induce different degrees of mobility in the protease inhibitor.

Tyrosine hydroxylase is the rate limiting enzyme in the biosynthesis of the catecholamine neurotransmitters. A model for its *in vitro* regulation was proposed based upon reversible conformational constraints held together by electrostatic interactions within the enzyme. Heparin activation affects these constraints through: 1) interaction with putative cationic amino acid residues of tyrosine hydroxylase that govern binding of cofactor (tetrahydrobiopterin) and 2) Novel non-specific cations, lysyltyrosine groups, inhibit the enzyme *in vitro*; thought to be bound to the enzyme *in vivo*, these contribute electrostatic constraints that govern binding of substrate, tyrosine; heparin reverse this inhibition. As in the heparin-antithrombin system, tyrosine hydroxylase exhibited the same dependence upon molecular weight of the mucopolysaccharide and the same specificity for heparin. A "low-capacity" form of tyrosine hydroxylase (defined by its low K_i for lysyl-tyrosine amide) was isolated by affinity-chromatography using the new inhibitor. 3) Polysaccharide heparin ($M_{Wt_{av}} \sim 8000$) is composed of a disaccharide repeat unit that consists mainly of 4-1 α -linked N-sulfated glucosamine 06 sulfate and 4-1 α -L iduronyl 02 sulfate residues. The unique antithrombin-modulating sequence differs in two repeat disaccharide units,... unsulfated L-iduronate α -4-Nacetylglucosamine 03 sulfate \rightarrow 1,4-glucuronate β -1, 4-N-sulfamino glucosamine 06 sulfate..., followed by the standard repeat unit to yield the binding hexasaccharide which is present in but ~ 30 percent of the isolated heparin molecules. Intrinsic, far ultraviolet circular dichroism spectra exhibit differences between the antithrombin-binding (active) and non-binding (inactive) heparin chains at 2200d and 6500d molecular weight, and at various pH's. Asymmetric dye binding sequences, although not exclusive to active heparin chains, were more stable in chains containing the active tetrasaccharide.

Far ultraviolet circular dichroism spectra of antithrombin-binding oligosaccharides, octa-to-octadecasaccharide, generated difference spectra that are equivalent to sequential disaccharide repeating units. Analyzed against optical models of unsulfated repeat units derived from α -D Nacetylglucosamine plus α -L iduronate glycoside or α -D Nacetylglucosamine plus β -D glucuronate glycoside, these spectra yielded the sequential positions of several disaccharides and the location of the second glucuronate moiety just preceding the second binding regions. A possible sequence from the fifth to the eleventh disaccharide is newly proposed.

Far ultraviolet circular dichroism and dye ligand-binding analysis were used as structural probes of the rare amino sugar [2-acetamido-2,6-dideoxy-D-glucosamine (D-quinovosamine) and 2-acetamido-2 deoxy-D-glucuronate]-containing microbial glycosaminoglycan from *Achromobacter georgiopolitani*: 1) the sugars appear to be linked primarily at the C_4 and C_1 positions of the pyranose ring; 2) a change in intrinsic circular dichroism with decreasing pH resembles that observed with β -glucuronosides; 3) the uronic acid moieties appear to

occur every other sugar in an extended sequence resembling that of the cellulose chain except for up to about 20 percent which occur as dimeric sites. 3) Sanfilippo heparan sulfates contain a major portion of oligosaccharides (from 2 to 20 saccharides), i.e., up to 73 percent for Sanfilippo A and 50 percent for Sanfilippo B. Hurler-1 mucopolysaccharides were about 25 percent in low molecular weight components. (See also Z01 MH 01038-09 LNC murine models for dihydropteridine reductase insufficiency).

Scientific Significance and Relevance to Public Mental Health: 1) Report selected to be presented at the XIth International Carbohydrate Symposium, Vancouver, Canada (August 22-29, 1982); abstract entitled "Circular dichroism spectroscopy of human antithrombin and its complexes with oligo- and polysaccharide heparins."

Modulation of protein function and enzyme systems by complex carbohydrates is implicated in all tissues including the brain. These biopolymers promote inhibitory, activation allosteric and/or release reactions in numerous systems. They are also implicated in the binding and exchange of simple and complex cations and water by interstitial tissue and cells because of their strong polyanionic character and/or viscosity. Heparin-like proteoglycans are present in cell membranes and are believed to be involved in cell-cell interactions and in fixing certain enzymes to the cell membrane. Results with the heparin-antithrombin system are the first to show that the duality of biological action of heparin binding regions obtains from the promoting of increasing degrees of change in the recognition region(s) of the protein. Furthermore, the second binding site of the modulator also brings about the close approximation of the inhibitor with the enzyme (thrombin) through a binding reaction with thrombin, so that the promotion of protein-protein reactions can now be visualized on a molecular basis. Multiple modulation reactions by a single effector can be a reflection of alteration of the recognition site, within a protein such as antithrombin, that leads to increasingly favorable geometry for the interaction of its inhibitory arginine residue with the active site of thrombin. An understanding of these reactions provides a basis for investigation of the modulation of tyrosine and tryptophan hydroxylases which regulate dopamine, norepinephrine and serotonin biosynthesis. 2) Findings were chosen for presentation at the XIth International Carbohydrate Symposium (Abstract entitled "Optical Properties of the Acidic Capsular Polysaccharide Isolated from *Achromobacter Georgiopolitani*", August 22-28, 1982). Other findings will be presented at the Annual Meeting of the Society for Complex Carbohydrates, September 22-24, 1982 (Abstract entitled "Circular Dichroism Spectroscopy and Analysis of Uronic Acid Sequences of Heparin Oligosaccharide Modulators").

Previous study of structure-function for heparin by Dr. Robert D. Rosenberg, utilizing his highly purified antithrombin-serine esterase systems, have made a major breakthrough in understanding how complex carbohydrate effect biological function. Current findings elucidate the molecular basis of the second mode of interaction of the oligosaccharide modulators, that which involves protein-protein apposition as well as protein activation, with a proposed sequence for the octadecasaccharide and 6500d heparin fragments. These oligosaccharide sequences provide potential for the development of oral anticoagulants or biomaterials for vessel replacement and may be involved in biosynthesis and release of the catechol amines of in the CNS. Sequence data provides rational for isolation of the second binding region fragment independent of the major binding

site. 3) Sanfilippo mucopolysaccharidoses involve accumulation of heparan sulfates, distinguishing cerebral pathology, atrophy in the central nervous tissue, and mental retardation, of which the initial etiology is unclear. Findings indicate that a large fraction of small oligosaccharides accumulate (along with higher molecular weight fractions) in the Sanfilippo disorder (but not to the same degree in Hurler). These can be isolated for testing as putative in vitro modulators or as effectors in a developmental biological system in order to explore specific causes for the pathophysiology of the mental retardation.

Proposed course:

1) Studies that measure the number of charged groups involved in the binding, full reports on the spectroscopy of the complexes of heparin fractions with the model polypeptides, and with antithrombin (which involve some computer analysis for curve resolution) are still pending. Investigation of the proposed involvement of a disulfide bond in the modulation of the antithrombin recognition region by the second heparin binding site will be initiated using Raman laser spectroscopy. Further efforts to elucidate the regulation of tyrosine hydroxylase by complex carbohydrates requires a more highly purified enzyme. Collaboration will be initiated when purified enzyme is available. 2) Sequence analysis (including techniques) of oligosaccharide modulators (including hexamer, hexadecamer and possible new model compounds), analysis of charge distribution in structure-function relations, and the theoretical predictions of ligand extrinsic circular dichroism will be continued. 3) Initial studies on the oligosaccharides of Sanfilippo mucopolysaccharidoses will be followed up with: 1) further isolations of discrete oligosaccharide fractions from 2 to 20 saccharides; 2) characterization by circular dichroism and other structural probes; 3) measurement of several possible biological activities such as modulation of CNS enzyme systems in vitro and as effector in a developmental neurobiological system.

Publications:

Stone, A. L.: Molecular Basis for the Activation of Tyrosine Hydroxylase by Heparin. In Lundblad, R.L., Brown, W.V., Mann, K.G., and Roberts, H.R. (Eds.) Chemistry and Biology of Heparin, Elsevier North Holland, Inc., pp. 143-156, 1981.

Stone, A. L., Beeler, D., Oosta, G., and Rosenberg, R. D.: Circular dichroism spectroscopy of heparin-antithrombin interactions. (PNAS, in press).

Stone, A. L.: Physicochemical characterization of heparin fractions. In Lundblad, R., Brown, W.V., Mann, K.G., and Roberts, H.R. (Eds.) Chemistry and Biology of Heparin. Elsevier North Holland, Inc., pp. 41-55, 1981.

* Due to reassignment from OD to LNB during the RIF period this Project Report was held over until permanent reassignment was permitted, so that the period covered extends back into the previous fiscal year to permit faithful reporting of the work and publications. This project is, in part, a continuation of Z01 MH 01036-09 LNC and Z01 MH 01038-09 LNC.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01031-14 LNC |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Conversion of Phenylalanine to Tyrosine | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Seymour Kaufman OTHER: Sheldon Milstien Michael Parniak Harvey Wilgus Berenice Gitomer Bruce Gomes Robert Phillips Frank Gold | Chief, Laboratory of Neurochemistry Research Chemist Visiting Associate Senior Staff Fellow Visiting Fellow Staff Fellow Staff Fellow Chemist | LNC NIMH LNC NIMH LNC NIMH LNC NIMH LNC NIMH LNC NIMH LNC NIMH LNC NIMH |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Neurochemistry | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 6.3 | PROFESSIONAL: 5.3 | OTHER: 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Phenylalanine hydroxylase</u> can be activated not only by its substrate, <u>phenylalanine</u> , but also by other large neutral <u>amino acids</u> . The enzyme can also be activated in vivo by <u>glucagon</u> and the activation is expressed in vivo. Phenylalanine hydroxylase is activated in <u>regenerating liver</u> by a process that probably also involves glucagon. The D-isomer of <u>tetrahydrobiopterin</u> inactivates pure phenylalanine hydroxylase. | | |

Project Description:

The objective of this research project is the detailed description of the enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components in the hydroxylation system. These components include phenylalanine hydroxylase, dihydropteridine reductase and tetrahydrobiopterin (BH_4).

One of the reasons why the regulation of this system is of special interest to neurochemists is that it can serve as a paradigm for the dynamic interaction between metabolic events in peripheral organs and the brain. When this interaction goes awry, as it does in classical phenylketonuria, it can lead to severe mental retardation.

Major Findings:

Previous work from this laboratory, and from others, has established that phenylalanine hydroxylase can be activated by its substrate, phenylalanine. We have now found that other large neutral amino acids such as methionine, norleucine, tryptophan, and even the product of the reaction, tyrosine, are able to activate the enzyme 25 to 30-fold. These novel amino acid activators not only activate by themselves, but they act synergistically with phenylalanine to increase the effectiveness of low concentrations of phenylalanine. In addition, we have found that some of these new amino acid activators are also able to serve as substrates for the enzyme that is activated by another mode, such as by lysolecithin. Activated phenylalanine hydroxylase, e.g., catalyzes the conversion of L-methionine to L-methionine sulfoxide, which represents, as far as we are aware, the first time that this reaction has been described with a mammalian enzyme. In contrast to L-methionine, which can serve as both an activator and as a substrate, D-methionine is unable to activate but is a weak substrate. These results provide strong evidence in favor of the idea that phenylalanine hydroxylase has a regulatory site that is distinct from its catalytic site.

We have previously found that phenylalanine hydroxylase is activated four-fold in vitro by a c-AMP dependent phosphorylation reaction. We also showed that the enzyme can be activated when rats are given a single dose of glucagon, the activation being measured in liver extracts. This in vivo activation probably involves a glucagon-mediated increase in the hepatic c-AMP levels, which, in turn, leads to increased phosphorylation of phenylalanine hydroxylase. Although these results suggested that the higher hydroxylase activity was actually expressed in vivo, they did not prove it. We have now shown that an intravenous injection of glucagon into rats leads to a prompt activation of phenylalanine hydroxylase that is expressed in vivo. The increased activity can be measured by a decrease in the steady-state concentration of blood phenylalanine and an increase in tyrosine during the time that phenylalanine is being infused into the animal at a constant rate. This is the first evidence that phenylalanine activity is under hormonal control in the whole organism.

Prompted by an earlier report in the literature that BH_4 levels in regenerating rat liver were elevated, we have attempted to confirm this finding with the HPLC assay for BH_4 . Although we were unable to confirm the claim that BH_4 levels are elevated, we did find that the activity of phenylalanine hydroxylase is increased about 250% in the regenerating liver. The fact that the BH_4 -dependent activity was increased whereas the

6-methyltetrahydropterin-dependent activity was unchanged, indicated that the increased phenylalanine hydroxylase activity was due to activation of the hydroxylase rather than to the synthesis of more hydroxylase molecules. Chromatographic analysis of the hydroxylase in extracts of regenerating liver on calcium phosphate - cellulose columns also indicated that the enzyme was activated. These results suggest that the enzyme in regenerating liver has been activated by a glucagon-mediated phosphorylation process.

We have previously shown that tetrahydrobiopterin (BH_4) is the naturally-occurring hydroxylation cofactor. Although this compound occurs in tissues as the L-isomer, the commonly used -chemically synthesized compound is a mixture of the D and L isomers (due to asymmetry of carbon atom 6). Recently these two isomers of BH_4 have been separated by HPLC. Comparing the activity of L- BH_4 and D- BH_4 with pure rat liver phenylalanine hydroxylase, we have found that although both isomers are active, the D-isomer leads to a rapid irreversible inactivation of the enzyme and that the inactivation depends on turnover of the enzyme.

Significance to Biomedical Research and Proposed Course:

The finding that phenylalanine hydroxylase can be activated by several large neutral amino acids and that they can act synergistically with phenylalanine to increase its effectiveness as an activator provides a more complete picture of how the activity of phenylalanine hydroxylase is regulated so that it keeps pace with demand.

One of the advantages of maintaining fine control over phenylalanine hydroxylase activity would be the protection that this would afford the developing brain. Even transient elevations of blood phenylalanine levels - such as those that would result from the ingestion of protein - could be damaging to the neonatal brain. Activation of phenylalanine hydroxylase by phenylalanine and by some of the other large neutral amino acids would provide one mechanism by which the hydroxylase would more promptly dispose of a bolus of phenylalanine.

Our demonstration that phenylalanine hydroxylase can be activated in vivo by glucagon provides another possible link between elevated blood level of phenylalanine and activation of the hydroxylase. Since amino acids including phenylalanine can elicit the release of glucagon, the activity of phenylalanine hydroxylase appears to be under both the indirect and direct control of phenylalanine and some of the other amino acids, the indirect effect being mediated by a glucagon-activated phosphorylation of the hydroxylase and the direct effect being mediated by occupancy by these amino acids of the regulatory site on the enzyme.

We plan to test whether the amino acids that can activate phenylalanine hydroxylase in vitro are able to do this in the whole organism. In addition, we plan to test whether activation by amino acids is mediated by glucagon.

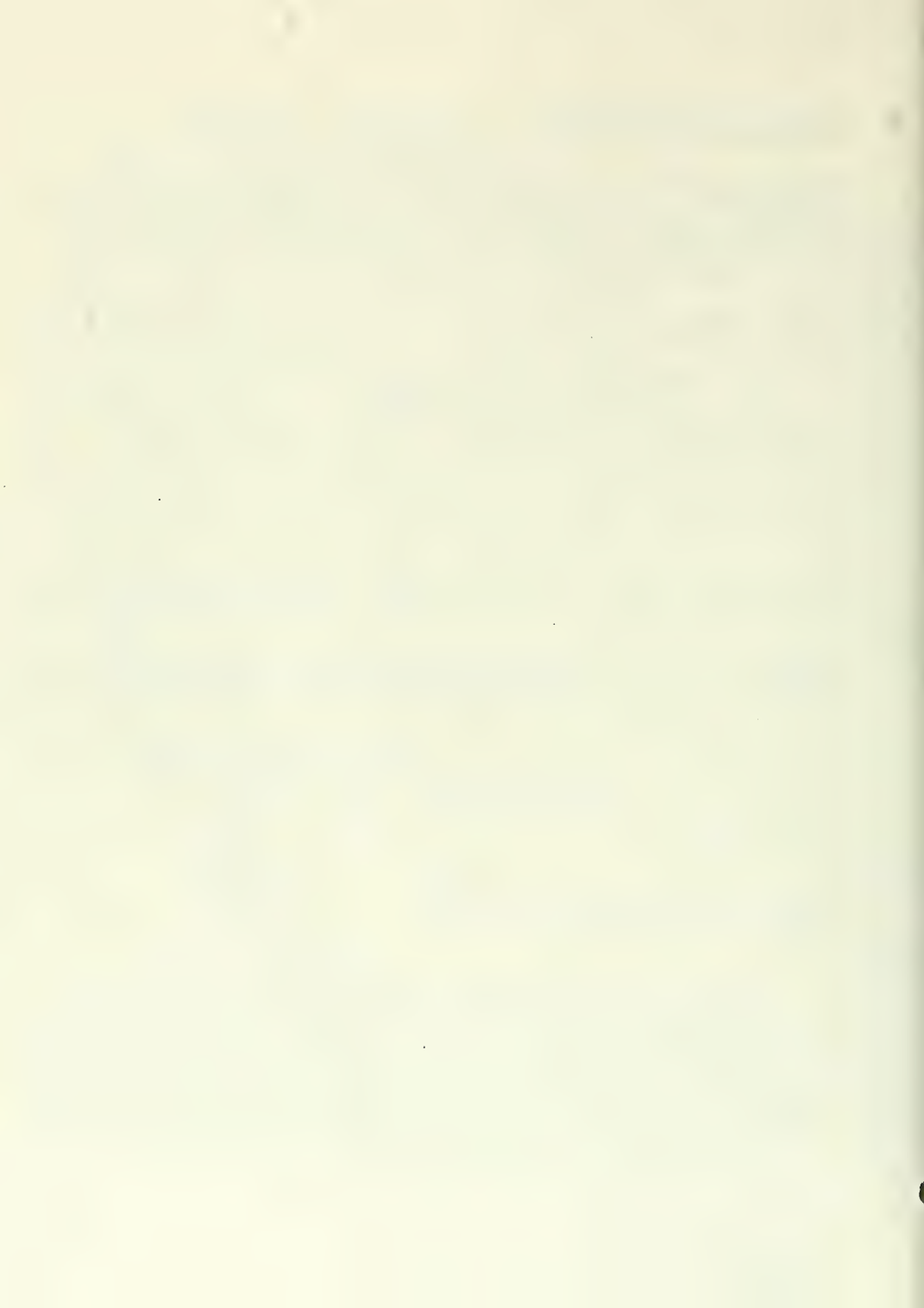
Probably related to the glucagon-mediated activation of phenylalanine hydroxylase is our finding that the enzyme in regenerating liver is in an activated state. It seems likely that this activation involves the release of glucagon that would be expected to follow the fall in blood sugar following partial hepatectomy. These results suggest that the activation of phenylalanine hydroxylase in the residual liver cells may serve as a compensatory response by the organism in an attempt to maintain a sufficient capacity for phenylalanine metabolism. Our plans are to see if this compensatory response is a general one. We also want to determine whether phenylalanine hydroxylase in regenerating liver is actually more highly phosphorylated.

The finding that the D-isomer of BH_4 is capable of inactivating phenylalanine hydroxylase in vitro has important implications for the use of pterins in the treatment of variant forms of PKU (See project number Z01 MH 01039-14 LNC). Currently, DL- BH_4 is being used in some of these patients as an alternative to the low phenylalanine diet to keep blood phenylalanine levels within the normal range. Our studies indicate that the long term use of DL- BH_4 may not be without some risk. We intend to study this possibility in whole animals.

Publications:

1. Parniak, M., and Kaufman, S.: Rat liver phenylalanine hydroxylase: Activation by sulfhydryl modification. J. Biol. Chem.; 256; 6876-6882; 1981.
2. Kaufman, S., Hasegawa, H., Wilgus, H., and Parniak, M.: The regulation of hepatic phenylalanine hydroxylase activity by phosphorylation and dephosphorylation. Cold Spring Harbor Conference on Cell Proliferation, 8; 1391-1406; 1981.
3. Hasegawa, H.; Parniak, M., and Kaufman, S.: Determination of the Phosphate Content of Purified Proteins. Analytical Biochemistry, 120; 360-364; 1982.
4. Kaufman, S. and Mason, K.: Novel Substrates and Activators for Rat Liver Phenylalanine Hydroxylase, Proceedings of the International Symposium on Oxygenases and Oxygen Metabolisms, in press, 1982.
5. Dhondt, J.-L., Kapatoss, G., Parniak, M., Wilgus, H., and Kaufman, S.: Biopterin metabolism and phenylalanine hydroxylase activity during early liver regeneration; Academic Press, Inc., Biochemical Biophysical Research Communications, 106, 786-793; June 15, 1982.
6. Mason, K., and Kaufman, S.: Specificity of Amino Acids as Activators and Substrates for Phenylalanine Hydroxylases, J. Biol. Chem., in press, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01032-14, LNC |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biosynthesis of Catecholamines | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div style="width: 40%;"> PI: Seymour Kaufman OTHER: Gregory Kapatos Bruce Gomes </div> <div style="width: 40%;"> Chief, Laboratory of Neurochemistry Staff Fellow Staff Fellow </div> <div style="width: 20%; text-align: right;"> LNC NIMH LNC NIMH LNC NIMH </div> </div> | | |
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| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Neurochemistry | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input checked="" type="checkbox"/> (c) NEITHER </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div> <input type="checkbox"/> (a1) MINORS </div> <div> <input type="checkbox"/> (a2) INTERVIEWS </div> </div> | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>The activity of <u>tyrosine hydroxylase</u>, the rate-limiting enzyme in the biosynthesis of <u>catecholamines</u>, is dependent upon a reduced unconjugated <u>pteridine, tetrahydrobiopterin</u>. We are actively pursuing enzyme purification procedures for tyrosine hydroxylase using as tissue sources both bovine striata and the adrenergic neuroblastoma, NIE115. Pteridine biosynthesis by rate brain, rat pineal glans, and adrenergic neuroblastoma, is being investigated in an attempt to elucidate the pathway by which pteridines are synthesized. The cellular mechanisms which regulate this synthesis, and the interactions between tyrosine hydroxylase and pteridines, are also being investigated.</p> <p><u>Discontinued.</u></p> | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01034-14 LNC | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Biochemical Basis of Skeletal Muscle Hypertrophy | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI: Seymour Kaufman</td> <td>Chief, Laboratory of Neurochemistry</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td>OTHER: Michael Bissell</td> <td>Senior Staff Fellow</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td>Rosanne Bailey</td> <td>Stay-In-School Student</td> <td>LNC</td> <td>NIMH</td> </tr> </table> | | | PI: Seymour Kaufman | Chief, Laboratory of Neurochemistry | LNC | NIMH | OTHER: Michael Bissell | Senior Staff Fellow | LNC | NIMH | Rosanne Bailey | Stay-In-School Student | LNC | NIMH |
| PI: Seymour Kaufman | Chief, Laboratory of Neurochemistry | LNC | NIMH | | | | | | | | | | | |
| OTHER: Michael Bissell | Senior Staff Fellow | LNC | NIMH | | | | | | | | | | | |
| Rosanne Bailey | Stay-In-School Student | LNC | NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neurochemistry | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.6 | PROFESSIONAL: 1.2 | OTHER: 0.4 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>The object of this work is the elucidation of the <u>biochemical mechanism of compensatory skeletal muscle hypertrophy</u>. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. Present topics of investigation include the relationship between <u>stretch-induced hypertrophy</u> and:</p> <ol style="list-style-type: none"> 1) <u>amino acid uptake and incorporation into muscle protein</u> 2) <u>cellular content of contractile protein</u> 3) <u>activity of the Na/K-dependent ATPase</u> 4) <u>characterization of substances with stretch-like activity</u> in this system. | | | | | | | | | | | | | | |

Project Description:

Two model systems for the study of this problem are currently in use in this laboratory. The *in vivo* "tenotomy model" system involves surgical section of the Achilles tendon of rats. Following this operation, the weight-bearing load is redistributed from the gastrocnemius to the two smaller synergist muscles, the soleus and plantaris, which rapidly hypertrophy. Using this system, we have seen significant increases in wet weight of the soleus and plantaris as early as four to six hours post-tenotomy.

The *in vitro* system consists of chick embryo myotubes cultured in monolayer on collagen-coated elastic silicone membranes attached to expandable nylon or teflon stretching frames. With the use of this model, it has been shown that skeletal myotubes respond to passive stretch by increased amino acid uptake (as measured by uptake of aminoisobutyric acid, AIB), increased incorporation of amino acids into, and accumulation of, both total cellular protein and myosin heavy chain. There is also an early increase in the V_{max} of the membrane Na/K-dependent ATPase, as measured by rubidium-86 uptake. This increase is not accompanied by change in the K_m of the enzyme nor in the number of ATPase units in the membrane, as measured by tritiated ouabain binding.

Major Findings:

The effects of stretch on amino acid uptake and incorporation into protein have been further characterized. They do not require serum or other "growth factors" in the medium. There is an approximately 30 minute lag period between the onset of stretch and the increases in these two processes. Furthermore, these stretch-induced increases do not persist to 60 minutes after the stretch is removed. The increases are insensitive to vincristine or colchicine, indicating that an intact microtubular system is not required. On the other hand, they are inhibited by ouabain after a 30 minute lag period, indicating a link between these processes and ATPase activation. Ouabain inhibits the ATPase "Na pump", depolarizing the cell membrane potential in this system. Inhibition is also observed by elevated extracellular potassium and tetrodotoxin, a specific binder to voltage-sensitive sodium channels, while amiloride, which inhibits passive sodium fluxes, has no effect. In other systems, it is known that AIB uptake is passively linked to the transmembrane concentration gradient of sodium, which results from "Na pump" activity. Passive stretch of myotubes may thus increase AIB uptake by activation of the Na/K-dependent ATPase, resulting in decreased intracellular sodium and hyperpolarization of the membrane potential.

Two complex biochemical mixtures have been found to have effects on AIB uptake and amino acid incorporation of unstretched myotube cultures that are similar to those seen with stretch. First, basal rates of AIB uptake are increased twofold by 15% serum when the myotubes are pre-incubated several hours in serum-free medium. This effect is dose-dependent and maximal at 15% serum. Stretching cultures in 15% serum results in no further increase in AIB uptake. The serum component involved is probably not insulin, since embryonic myotubes are insensitive to insulin.

Secondly, aqueous extracts of hypertrophied hindlimb muscles from rats 19 to 39 hours post-tenotomy cause increases in AIB uptake and amino acid incorporation when added to unstretched myotube cultures. Similar extracts from the corresponding nonhypertrophied muscles on the unoperated contralateral hindlimbs of the same animals do not show this effect when added to the cultures. Further characterization of the substance or substances responsible for this "stretch-like" activity in myotube cultures is currently underway.

Significance to Biomedical Research and to the Program of the Institute:

The immediate applications of this work are likely to be in exercise physiology, cardiovascular physiology, and neuromuscular pathophysiology. Fundamental understanding of this basic process will conceivably help advance one or more of these areas in a significant way. In addition, and in a broader sense, this sequence of biochemical events leading from the stimulus of increased mechanical stretch to a structural change, i.e., increased contractile protein content, may be regarded as a form of "cellular learning." As such, certain of its features may prove to be applicable to the study of structural adaptation of other tissues under conditions of increased physiological demand.

Proposed Course:

At the current level of effort, completion of the lines of experimentation now planned will require three or more years.

Publications:

1. Vandeburgh, H. H., Kaufman, S.: Stretch-induced growth of skeletal myotubes correlates with activation of the sodium pump; J. Cell Physiol.; 109; 205-214; 1981.
2. Vandeburgh, H. H., Kaufman, S.: Absence of a serum requirement for stretch-induced amino acid transport in skeletal myotubes and the role of membrane potential alterations; J. Biol. Chem.; 1982 (in press).
3. Vandeburgh, H. H., and Kaufman, S.: Short and long term modification of skeletal muscle Na pump activity. Effects on muscle protein turnover. In Mechanism of Muscle Adaptation to Functional Requirements (R. Guba, G. Marechal, O. Takacs, Eds.), Akademiai Kiado, Publishing House of the Hungarian Academy of Science, Budapest, pp. 291-304; 1980.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01035-14 LNC | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Process of Lysogeny | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table> <tr> <td>PI:</td> <td>Howard Nash</td> <td>Medical Research (Officer)</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td>OTHER:</td> <td>Brenda Lange-Gustafson</td> <td>Staff Fellow</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Nancy Craig</td> <td>Staff Fellow</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Carol Robertson</td> <td>Biologist</td> <td>LNC</td> <td>NIMH</td> </tr> </table> | | | PI: | Howard Nash | Medical Research (Officer) | LNC | NIMH | OTHER: | Brenda Lange-Gustafson | Staff Fellow | LNC | NIMH | | Nancy Craig | Staff Fellow | LNC | NIMH | | Carol Robertson | Biologist | LNC | NIMH |
| PI: | Howard Nash | Medical Research (Officer) | LNC | NIMH | | | | | | | | | | | | | | | | | | |
| OTHER: | Brenda Lange-Gustafson | Staff Fellow | LNC | NIMH | | | | | | | | | | | | | | | | | | |
| | Nancy Craig | Staff Fellow | LNC | NIMH | | | | | | | | | | | | | | | | | | |
| | Carol Robertson | Biologist | LNC | NIMH | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Division of Biology and Medicine, Brown University; Laboratory of Molecular Genetics, NICHD; Laboratory of Molecular Biology, NCI; Laboratory of Molecular Biology, NIAMDD | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neurochemistry | | | | | | | | | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | |
| <p>The organization of <u>synapsis</u> during <u>integrative recombination</u> of <u>bacteriophage lambda</u> has been probed by measurement of the change in <u>supercoiling</u> that accompanies integration. A unique loss of two superhelical turns is found, indicating the two recombining DNA double helices are brought together during synapsis in a tight, ordered structure. The role of <u>topoisomerases</u> in recombination has been confirmed by finding specific topoisomerase cleavage produced by a purified recombination protein, Int, at the crossover locus. Although Int protein normally requires a <u>host protein</u> to promote integration, a variant Int protein has been characterized that accomplishes recombination by itself.</p> | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

The organization of genetic information can be modified through rearrangement of blocks of DNA sequence. Such rearrangement, or recombination, can have a major impact on the expression of genetic information. For example, the expression of antibody genes requires a specific set of genetic recombinations during the differentiation of antibody producing cells. Similarly, the expression of alternate surface flagellar antigens in the bacterial pathogen *Salmonella* depends on the rearrangement of specific controlling DNA sequences. The site specific recombination that is involved in lysogeny of bacteriophage lambda is the most completely characterized of this class of DNA rearrangements. During lysogeny, DNA of the lambda virus is inserted into the chromosome of its *E. coli* host. This laboratory was the first to demonstrate that this recombination could be carried out in a cell-free system. We are now engaged in an in-depth study of the biochemical mechanism of lambda integrative recombination.

A fundamental question concerning all recombination is the organization of synapsis, the process of bringing two DNA double helixes together at the start of recombination. In order to find out about the intimacy and tightness of synapsis, we have measured the precise change in supercoiling when closed circular DNA substrates undergo lambda integrative recombination *in vitro*. We reason that if synapsis involves a highly ordered structure, there will be little chance for random DNA unwinding between the time of the initial breakage of parental DNA and the final ligation step that rejoins the broken ends to new partners. Conversely, loose synaptic structures, in which DNAs are brought into only rough approximation, should result in recombinants that have lost variable degrees of supercoiling. We measured the precise change in supercoiling that accompanies *in vitro* lambda site specific recombination by the following strategem. We constructed plasmids that contain the two specialized sites for lambda integration on the same circle of DNA; these sites are oriented such that a recombination between them inverts one arc of the circle with respect to the other arc. The product DNAs thus have the same size and nucleotide composition as the substrate; any change in electrophoretic mobility can be directly ascribed to a change in supercoiling. A complication of this approach is the production of knotted rather than simple circular, recombinant products. We found conditions where knotting is minimal and purified the simple circular products away from the knots by a second cycle of electrophoresis. The results show that essentially all recombinants have undergone a unique change in supercoiling, being relaxed by two superhelical turns compared to the substrate. This strongly implies that synapsis is a highly ordered process that permits only limited movement of the DNA chains with respect to one another during the exchange step of recombination. It should be pointed out that the loss of two superhelical turns agrees precisely with the value predicted by a hypothetical model for synapsis and strand exchange that this laboratory first published several years ago.

The same experimental strategy was applied to the site specific recombination that normally excises the integrated viral DNA. Excisive recombination uses the same two proteins required for integrative recombination and additionally requires a third protein, Xis, that has been purified and characterized in the laboratory of S. Wickner. For excisive recombination, we find precisely the same unique loss of two superhelical turns as found for integrative recombination. This indicates that Xis protein does not grossly alter the integrative recombination mechanism but simply adjusts the recombination apparatus to accommodate the sites that bound the integrated virus.

A second fundamental question about the mechanism of site specific recombination concerns that manner by which DNA strands are broken and rejoined during a crossover.

From the apparent absence of a high energy cofactor requirement for in vitro recombination, we have concluded that crossing over does not proceed by the separate action of a nuclease and a ligase. Instead, these steps must be coupled in a manner similar to that described for a class of enzymes, topoisomerases, that reversibly cleave DNA by forming covalent enzyme-DNA intermediates. Indeed, we have found that one of the proteins required for recombination, Int, has a topoisomerase activity that is uncoupled from recombination. In order to assess the relevance of this activity to recombination, we have determined the DNA sequence specificity of Int topoisomerase. End labelled restriction fragments, 50 to 400 base pairs long were incubated with Int, deproteinized, and electrophoresed. Autoradiography of the high resolution electrophoresis gels show that a small fraction of the labelled DNA is cleaved by Int precisely at the nucleotide position where integrative recombination crossovers normally occur. This cleavage is like that expected from a topoisomerase in that: 1) one end of the break is covalently linked to protein and 2) the break can be reversibly resealed in the absence of additional cofactors. This finding confirms the hypothesis that crossing over is accomplished by a specific topoisomerase.

In addition to the virus encoded Int protein, integrative recombination also requires a host encoded protein, Integration Host Factor (IHF). Since Int appears to be responsible for the breakage and reunion steps in recombination, we believe that IHF serves a secondary role in recombination. This hypothesis is supported by in vitro studies of Int-h, a variant Int protein that was selected for ability to lysogenize E.coli that carry mutations in the genes encoding IHF. We have purified the variant Int protein to homogeneity and have demonstrated that this altered protein does carry out integrative recombination in the complete absence of IHF. This eliminates models that invoke altered interactions between Int-h and altered IHF as the basis of the Int-h phenotype. Conversely, these results show that the Int protein carries all the essential elements required for recombination.

Significance to Biomedical Research and Proposed Course:

The present studies continue to advance our understanding of a fundamental biological process - the recombination of genetic information. Our study of topological changes induced by recombination is essentially complete and will be prepared for publication. The characterization of specific cleavages produced by Int protein will focus on the chemical nature of the covalent protein-nucleic acid joint. We plan to study the details of interaction between Int-h and DNA so as to infer the alterations that obviate the requirement for IHF.

Publications:

1. Nash, H. A., Site-Specific Recombination Protein of Phage Lambda, The Enzymes 14, Vol. XIV, 471-480; 1981.
2. Nash, H. A. and Robertson, C. A., Purification and Properties of the E. coli Protein Factor Required for Lambda Recombination. J. Biol. Chem.; 256 (17); 9246-9253; September 10, 1981.
3. Nash, H. A.: Integration and Excision of Bacteriophage Lambda: The Mechanism of Conservative Site Specific Recombination. Ann. Rev. Genet.; 15; 143-167; 1981.

4. Pollock, T. J. and Nash, H. A.: Studies on the role of DNA supercoiling in lambda integrative recombination. In H. W. Woolhouse (Ed.): Biological Consequence of DNA Structure and Rearrangement, John Innes Charity, Norwich, England, in press, 1982.
5. Nash, H. A.: Purification and Properties of the Bacteriophage Lambda int Protein: Recombinant DNA, Methods in Enzymology. In S. P. Colowick and N. O. Kaplan (Eds.); Academic Press, Inc., New York, New York, in press, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01037-14 LNC | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The Role of the Cell Membrane in Cellular Organization, A Molecular Study. | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: David M. Neville, Jr.</td> <td style="width: 40%;">Chief, Section on Biophys Chem.</td> <td style="width: 20%;">LNC</td> <td style="width: 20%;">NIMH</td> </tr> <tr> <td>OTHER: Richard Youle</td> <td>Staff Fellow</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td>Steven Esworthy</td> <td>Staff Fellow</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td>Ateeq Ahmad</td> <td>Visiting Associate</td> <td>LNC</td> <td>NIMH</td> </tr> </table> | | | PI: David M. Neville, Jr. | Chief, Section on Biophys Chem. | LNC | NIMH | OTHER: Richard Youle | Staff Fellow | LNC | NIMH | Steven Esworthy | Staff Fellow | LNC | NIMH | Ateeq Ahmad | Visiting Associate | LNC | NIMH |
| PI: David M. Neville, Jr. | Chief, Section on Biophys Chem. | LNC | NIMH | | | | | | | | | | | | | | | |
| OTHER: Richard Youle | Staff Fellow | LNC | NIMH | | | | | | | | | | | | | | | |
| Steven Esworthy | Staff Fellow | LNC | NIMH | | | | | | | | | | | | | | | |
| Ateeq Ahmad | Visiting Associate | LNC | NIMH | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Genetics and Biochemistry Branch, NIAMDD Minnesota Bone Marrow Transplantation Group | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neurochemistry | | | | | | | | | | | | | | | | | | |
| SECTION Biophysical Chemistry | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 5.0 | PROFESSIONAL: 4.0 | OTHER: 1.0 | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, <u>plasma membrane receptor</u> mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein hybrids containing the active fragment of a <u>toxin</u> and another receptor specific binding protein. Such artificial protein hybrids may have value as a new class of pharmacologic reagents. Monoclonal antibody ricin conjugates directed against T cells effectively eliminate these cells from donor bone marrow permitting <u>bone marrow transplants</u> free from <u>graft versus host disease</u> . This will provide a new treatment for <u>leukemia</u> , <u>aplastic anemia</u> and <u>autimmune diseases</u> . | | | | | | | | | | | | | | | | | | |

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to synthesize a new class of pharmacologic reagents which permit the modulation of specific cell types in a manner unobtainable with conventional reagents. This is being accomplished by the synthesis of artificial protein hybrids which utilize the process of receptor-mediated protein transport.

A wide variety of proteins are capable of entering cells by receptor-mediated transport processes. Having gained entry these proteins are directed to specific cellular compartments where they exert either a physiological or pathological function.

In general it appears that only a discrete portion of these proteins contain the receptor binding activity which is involved in the entry process while another portion of the protein performs the intracellular function. Therefore, it is possible to split and reassemble two such proteins with a new combination of receptor entry specificity and intracellular function. Such proteins we call artificial hybrid proteins, and previous reports from this laboratory have suggested that such hybrids should have utility both as probes of entry processes and as a new class of pharmacologic reagents with tailor made receptor and therefore cell type specificity.

Major Findings:

Anti T cell monoclonal antibody ricin conjugates in the presence of lactose reduce the T cell population of murine donor bone marrow by greater than 95% without affecting the viability of the marrow stem cell population. Thus, the immunologic memory of the donor marrow is lost and this marrow can be infused into an irradiated recipient and a new marrow will be reconstituted. Graft versus host disease does not occur because the T cells which carry the memory of self have been eliminated.

A similar anti-T cell conjugate directed at human T cells has been constructed and assayed and has similar properties.

Significance to Biomedical Research:

The major drawback to bone marrow transplantation is graft versus host disease. The newly developed anti-human T cell monoclonal antibody ricin conjugates will now be tested clinically to determine if this reagent will permit bone marrow transplantation across major histocompatibility barriers in humans. If this is successful, cures for a variety of leukemias, aplastic anemia, and fatal autoimmune diseases will be at hand by providing a new marrow to an irradiated recipient. Currently transplants are limited to those individuals having an identical twin or an HLA matched sibling (one out of four chance for each sibling.)

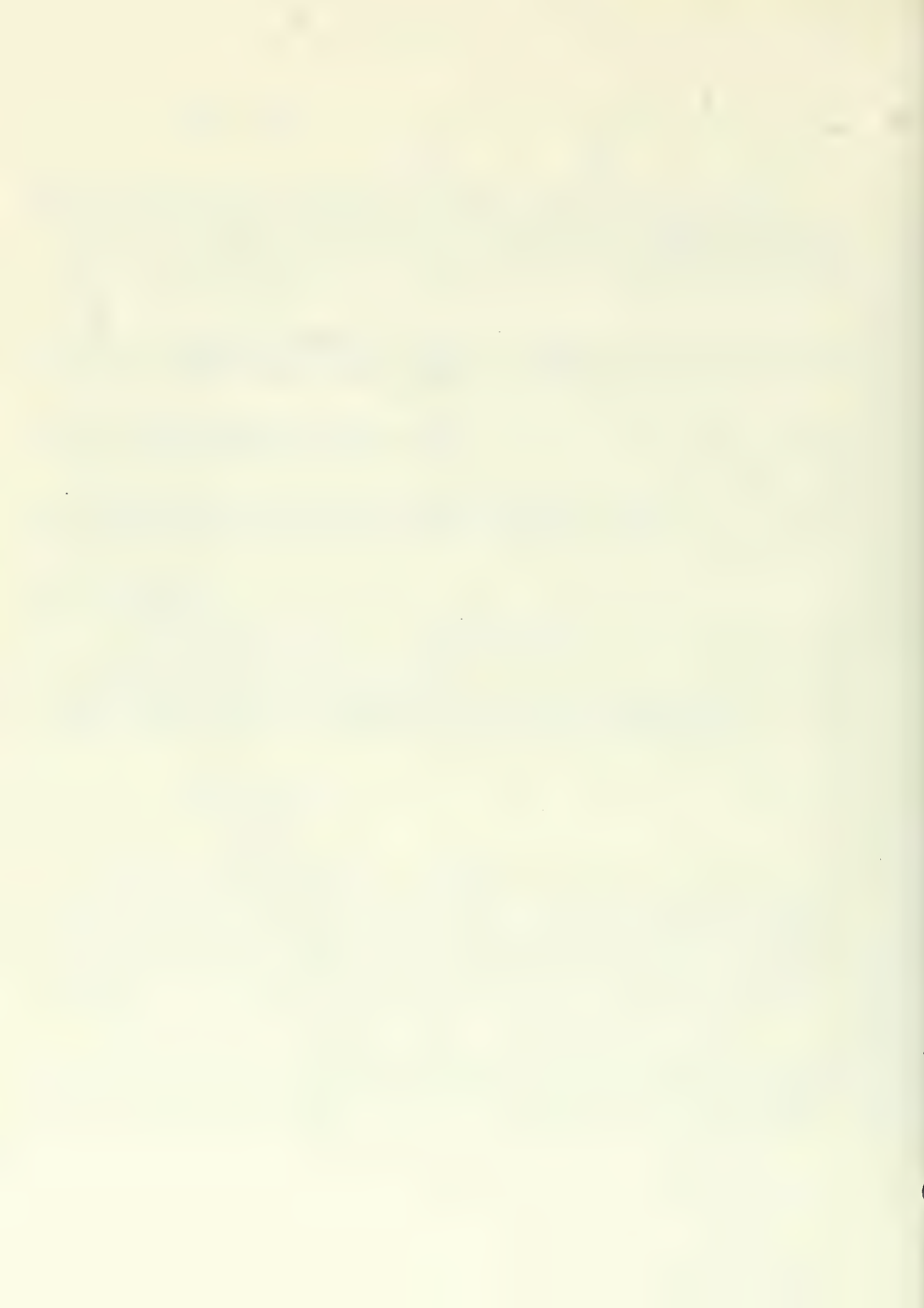
There is considerable speculation that certain subgroups of depressive disorders or schizophrenia might have an autoimmune etiology. The most direct test of this hypothesis is to include into the clinical bone marrow transplant protocol patients suffering from a disease requiring marrow transplantation and prior psychoses, and to follow the mental status after marrow transplantation.

Proposed Course of Project:

Clinical trials are scheduled to begin in August 1982. Efforts are being made to develop hybrid protein reagents which can be used in vivo to either erase the memory of the immune system or selectively kill off other cellular populations such as benign or malignant tumors.

Publications:

1. Neville, D.M., Jr., Murray, G.J., Heagy, W., and Youle, R.J.: Directed entry of hybrid proteins into cells via alternate receptors. In: Middebrook, J.L. and Kohn, L.D. (eds.), Receptor-Mediated Binding and Internalization of Toxins and Hormones. Academic Press, New York, 1981, pp. 329-336.
2. Neville, D.M., Jr., and Youle, R.J.: Monoclonal antibody-ricin or ricin A chain hybrids. Kinetic analysis of cell killing for tumor therapy. Immunol. Rev., 62: 75-91, 1982.
3. Youle, R.J. and Neville, D.M., Jr.: Kinetics of protein synthesis inactivation by ricin-antio thy 1.1 monoclonal antibody hybrids: Role of the ricin B subunit demonstrated by reconstitution. J. Biol. Chem., 257: 1598-1601, 1982.
4. Valleria, D.A., Youle, R.J., Neville, D.M., Jr., and Kersey, J.H.: Bone marrow transplantation across major histocompatibility barriers. III. Protection of mice from lethal GVHD by pretreatment of donor cells with monoclonal anti-thy 1.2 coupled to ricin. J. Exp. Med., 155: 949-954, 1982.
5. Youle, R.J. and Neville, D.M., Jr.: Hybrid proteins used to study the mechanism of toxin entrance into cells. In: Weinstein, L. and Fields, B.N. (eds.), Seminars in Infectious Disease. Thieme-Stratton, Inc., 1982, Vol. IV (Bacterial Vaccines), pp. 86-88.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01038-1 LNC |
| PERIOD COVERED October 1, 1981 through September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Phenylketonuria and Other Diseases Caused by Defects in Biopterin-Dependent Enzymes | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Seymour Kaufman Chief, Laboratory of Neurochemistry LNC NIMH OTHER: Gregory Kapatos Staff Fellow LNC NIMH Rosanna Bailey Stay-In-School Student LNC NIMH | | |
| COOPERATING UNITS (if any) Section on Human Biochemical Genetics, National Institute of Child Health and Human Development | | |
| LAB/BRANCH Laboratory of Neurochemistry | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 0.6 | OTHER: 0.4 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Substantial amounts of <u>tetrahydrobiopterin</u> and <u>6-methyltetrahydropterin</u> can be detected in <u>CSF</u> when these pterins are given <u>peripherally</u> to patients with <u>hyperphenylalaninemia</u> due to defective <u>biopterin</u> synthesis. Our results suggest that administration of either of these pterins in proper doses may prove to be a treatment not only for the impaired peripheral phenylalanine metabolism, but also for the <u>neurological disorders</u> that are characteristic of the variant forms of hyperphenylalaninemia due to defective <u>BH₄</u> synthesis or metabolism. With respect to more general applications, administration of large doses of tetrahydropterins could provide a novel way to increase the biosynthesis of <u>catecholamines</u> and <u>serotonin</u> in both the brain and periphery, and could therefore be useful in the treatment of other human disorders suspected to result from deficits in biogenic amine biosynthesis, such as in <u>Parkinson's disease</u> and certain forms of <u>depression</u> . | | |

Project Description:

In 1975, cases of hyperphenylalaninemia were reported in which neurological disorders persist despite dietary control of phenylalanine blood levels. Subsequently, variant forms of phenylketonuria (PKU) or hyperphenylalaninemia were described by our laboratory in which the defect in the phenylalanine hydroxylase system is not in phenylalanine hydroxylase, itself, as it is in classic PKU, but rather in dihydropteridine reductase or in an enzyme involved in the biosynthesis of tetrahydrobiopterin (BH_4). Dihydropteridine reductase functions to maintain BH_4 in its functional tetrahydro form while BH_4 is an essential coenzyme. Both of these variants are therefore characterized by a marked deficiency of BH_4 . Since, as previous work in this laboratory had shown, this pterin is an essential coenzyme not only for phenylalanine hydroxylase, but also for tyrosine and tryptophan hydroxylases, patients lacking BH_4 suffer from defects in the synthesis of the neurotransmitters, dopamine, norepinephrine, epinephrine and serotonin in both the peripheral and central nervous systems, as well as from an impaired ability to hydroxylate phenylalanine in the liver. Indeed, to our knowledge, these patients are the only population presently available whose neurological dysfunctions can unequivocally be attributed to a genetic defect in biogenic monoamine synthesis which does not appear to involve irreversible cell loss. These patients might therefore be considered as models for other nondegenerative neurological diseases, the etiology of which is believed to involve aberrations in biogenic monoamine metabolism.

Current therapy for these variant forms of hyperphenylalaninemia consists of restriction of phenylalanine intake and administration of the hydroxylated amino acid precursors of catecholamines and serotonin, 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan, respectively, in conjunction with inhibition of peripheral aromatic amino acid decarboxylation with carbidopa. Although administration of BH_4 to these patients, especially to those with a defect in BH_4 biosynthesis, might also appear to be a reasonable therapy, the reports that this pterin does not readily enter the brain from the periphery made it seem unlikely that this treatment would prevent the neurological damage that characterizes these diseases.

Major Findings:

We have recently shown that tetrahydropterins such as BH_4 and 6-methyltetrahydropterin ($6MPH_4$), a synthetic analogue of BH_4 with high hydroxylation cofactor activity, when administered peripherally to rats in large doses, are able to cross the blood brain barrier. The positive results of our animal studies encouraged us to try to assess the ability of these pterins to cross the blood brain barrier in patients with hyperphenylalaninemia due to defective biosynthesis of BH_4 .

The oral administration of 20 mg/kg/day of BH_4 to one of these patients was found to increase CSF biopterin concentrations by 20-fold 2.5 hours after the BH_4 had been given. Three hours after the intravenous administration of 20 mg/kg of $6MPH_4$ to the second patient, large amounts of the pterin were also detected in samples of CSF. These BH_4 and $6MPH_4$ levels in CSF are comparable to those found in rat brains after they had been given similar intraperitoneal doses of these pterins.

Our results show that, contrary to currently accepted notions, tetrahydropterins such as BH_4 and $6MPH_4$, when given at a proper dose, do indeed cross the blood brain barrier. Our present findings extend to humans, therefore, our previous results with rats. With both species, the pterin with the more hydrophobic side chain, $6MPH_4$, enters the brain more readily than does the pterin with the less hydrophobic side chain, BH_4 . With respect to the

present findings that significant amounts of BH_4 and $6MPH_4$ can be found in CSF when higher doses of these pterins are given, preliminary studies suggest that the increased pterin levels are capable of stimulating the activity of brain tyrosine and tryptophan hydroxylases. There are also indications that neurological symptoms improve dramatically when larger doses of these tetrahydropterins are administered to some of these patients, suggesting that administration of either BH_4 or $6MPH_4$ (or a related tetrahydropterin with high hydroxylation cofactor activity) may prove to be adequate therapy for variant forms of PKU caused by defects in BH_4 synthesis. We should emphasize that pterin administration as used here may prove to have important advantages over neurotransmitter replacement as a therapy for these variant forms of PKU. Thus, even though administration of the neurotransmitter precursors is clearly beneficial, this treatment bypasses any regulatory mechanisms that normally may be mediated by coupling between nerve stimulation and neurotransmitter secretion. Also, neurotransmitter precursors cannot substitute for BH_4 in all the roles that this pterin might play in the organism. In terms of a more general application, administration of large doses of tetrahydropterins could provide a novel way to increase the biosynthesis of catecholamines and serotonin in both the brain and periphery, and could therefore be useful in the treatment of human disorders suspected to result from deficits in biogenic amine biosynthesis, such as in Parkinson's disease and certain forms of depression.

Within the last year our clinical screening program has discovered what appears to be a new variant form of PKU. This patient, an infant female, has a persistent and profound impairment of peripheral BH_4 synthesis which, in contrast with that of other BH_4 -deficient patients, and as evidenced by normal values for CSF pterins, biogenic monoamine metabolites and the absence of neurodevelopmental abnormalities, is not manifested in the central nervous system. These observations are highly significant to mental health research because they suggest that different isozymes of at least one enzyme that is involved in the synthesis of BH_4 may be different in neural and non-neural cell types. If this does indeed turn out to be the case, we might also predict that humans exist who have normal peripheral BH_4 synthesis but who synthesize either too little or too much BH_4 in the central nervous system, and therefore have similar alterations in biogenic monoamine synthesis.

Significance to Biomedical Research and Future Course:

The significance of these findings to biomedical research is that they provide a rational basis for the treatment of hyperphenylalaninemia due to defects in BH_4 synthesis and metabolism. Furthermore, the administration of either BH_4 or 6-methyltetrahydropterin or 6,7-dimethyltetrahydropterin at the doses that we have established are adequate to significantly increase pterin levels in the brain may also be useful in the treatment of other human disorders that are suspected to result from deficits in biogenic amine biosynthesis, such as Parkinson's disease and certain forms of depression. We intend to explore these possibilities.

Publications:

1. Kaufman, S., Kapatos, G., McInnes, R.R., Schulman, J.D., and Rizzo, W.B. The use of tetrahydropterins in the treatment of hyperphenylalanemia due to defective synthesis of tetrahydrobiopterin: Evidence that peripherally administered tetrahydropterins enter the brain. *Pediatrics*, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01039-1. LNC | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">October 1, 1981 through September 30, 1982</p> | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Pteridine Biosynthesis | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Seymour Kaufman</td> <td style="width: 40%;">Chief, Laboratory of Neurochemistry</td> <td style="width: 20%;">LNC</td> <td style="width: 20%;">NIMH</td> </tr> <tr> <td>OTHER: Gregory Kapatos</td> <td>Staff Fellow</td> <td>LNC</td> <td>NIMH</td> </tr> <tr> <td>Sheldon Milstien</td> <td>Research Chemist</td> <td>LNC</td> <td>NIMH</td> </tr> </table> | | | PI: Seymour Kaufman | Chief, Laboratory of Neurochemistry | LNC | NIMH | OTHER: Gregory Kapatos | Staff Fellow | LNC | NIMH | Sheldon Milstien | Research Chemist | LNC | NIMH |
| PI: Seymour Kaufman | Chief, Laboratory of Neurochemistry | LNC | NIMH | | | | | | | | | | | |
| OTHER: Gregory Kapatos | Staff Fellow | LNC | NIMH | | | | | | | | | | | |
| Sheldon Milstien | Research Chemist | LNC | NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">Laboratory of Neurobiology, National Institute of Child Health & Human Development</p> | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Laboratory of Neurochemistry</p> | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</p> | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <p style="text-align: center;">1.1</p> | PROFESSIONAL: <p style="text-align: center;">1.1</p> | OTHER: <p style="text-align: center;">0.0</p> | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES </div> <div> <input checked="" type="checkbox"/> (c) NEITHER </div> </div> | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> With the use of numerous preparations, including a cell-free system from rat <u>brain</u>, rat <u>pineal gland</u> in organ culture, and the <u>adrenergic neuroblastoma</u> N1E115, we are investigating the <u>tetrahydrobiopterin (BH₄) biosynthetic pathway</u> and the cellular processes which <u>regulate</u> this synthesis. We have also begun preliminary investigations, with the use of a <u>regenerating</u> rat liver preparation and the <u>developing</u> rat brain and pineal gland, into the possible role of BH₄ in cellular <u>proliferation</u>. </p> | | | | | | | | | | | | | | |

Tetrahydrobiopterin (BH_4) is a naturally occurring reducing agent; the only well-established role for this compound is as a cofactor in the enzymatic hydroxylation of aromatic amino acids by the family of three pterin-dependent mixed-function monooxygenases which are commonly referred to as phenylalanine, tyrosine, and tryptophan hydroxylases. These enzymes are rate-limiting in the catabolism of phenylalanine, and the synthesis of catecholamines and indoleamines, respectively. Surprisingly, although BH_4 was discovered over thirty years ago, its biosynthetic pathway has not been unequivocally determined. Similarly, while there is no doubt that BH_4 biosynthesis is regulated within the cell, no reports of homeostatic mechanisms have been reported.

Biopterin Biosynthesis by Rat Brain

We have described a method for the determination of [^{14}C] biopterin biosynthesis from [^{14}C] guanosine-5'-triphosphate by a desalted preparation from rat striatum which is based upon sequential reverse-phase and cation-exchange high performance liquid chromatography. Synthesis of reduced forms of biopterin by this preparation was found to be dependent upon enzymatic activity, guanosine-5'-triphosphate, magnesium ions, and a pyridine nucleotide. As demonstrated by the technique of isotope dilution, isotope trapping, sepiapterin was found to be an intermediate in biopterin biosynthesis that is catalysed by the striatal extract. Rat brain was also shown to synthesize biopterin *in vivo* from intraventricularly administered [^{14}C] guanosine or sepiapterin. In contrast to previous reports, the biosynthesis of biopterin by rat brain does not, therefore, appear to differ from that occurring in other, non-neural tissues.

Regulation of Biopterin Biosynthesis:

Pineal Gland

The importance of the pineal gland and its indoleamine hormone, melatonin, to the entrainment of human circadian rhythms, and of normal rhythms to mental health, have only recently been determined. Because BH_4 is required for the synthesis of the serotonin, precursor of melatonin, we have investigated the biosynthesis of BH_4 in the rat pineal gland in the hope of obtaining insight into what role BH_4 plays, if any, in the production of melatonin rhythms. Many aspects of pineal function, including melatonin production, are neurally regulated by an adrenergic-cyclic AMP mechanism. We have demonstrated that the biosynthesis of pineal BH_4 is regulated by a similar mechanism. Stimulation of the cultured pineal gland with beta-adrenergic receptor agonists or cyclic-AMP analogues was found to inhibit BH_4 synthesis and decrease BH_4 levels in the gland. This response was blocked by beta-adrenergic receptor antagonists and was not mimicked by alpha-adrenergic agonists. Because, in contrast, the synthesis of the pineal hormone, melatonin, is stimulated by an adrenergic-cyclic-AMP mechanism, we sought to determine whether the decline in BH_4 and the production of the melatonin rhythm were related. Incubation of glands with sepiapterin, so as to block the receptor-mediated decline in BH_4 , did not affect the induction of the enzyme, N-acetyltransferase, which is rate-limiting in melatonin synthesis. Similarly, incubation of glands with melatonin in the absence of adrenergic receptor stimulation did not alter BH_4 levels. The decline in BH_4 levels in response to adrenergic stimulation does not, therefore, appear to be directly related to the melatonin rhythm. The mechanism or physiological importance of this adrenergic-cyclic AMP-dependent regulation of pineal BH_4 synthesis requires further research. This is, however, the first indication that the biosynthesis of BH_4 in any tissue is under regulatory control.

Neuroblastoma

The adrenergic neuroblastoma N1E115 was found to synthesize two pterins, neopterin and BH₄. The biosynthesis of these pterins was determined by incubation of the cells with [¹⁴C] guanosine. Initial rates of synthesis of [¹⁴C] neopterin and [¹⁴C] biopterin were inhibited in a concentration-dependent manner by addition of exogenous BH₄ to the culture medium. This decrease in synthesis was not the result of either: 1) a decline in the labeling of [¹⁴C] guanosine-5'-triphosphate, from [¹⁴C] guanosine, 2) an increase in [¹⁴C] pterin degradation or 3) a stimulation of [¹⁴C] pterin efflux into the culture medium. We conclude from these data that the synthesis of both neopterin and BH₄ in this cell line is inhibited by BH₄. These experiments support recent observations made in this laboratory which showed that the elevated neopterin levels present in the urine, plasma, and CSF of humans who are genetically defective in biopterin synthesis can be rapidly and dramatically decreased by administration of tetrahydropterins. The chemical synthesis of reduced pterins which are not cofactors for pterin-dependent hydroxylases but which are capable of inhibiting BH₄ synthesis may therefore usher in a new class of biogenic monoamine biosynthesis inhibitors.

Biopterin and Cell Proliferation:

The nearly ubiquitous distribution of BH₄ in animal tissues and some of the enzymes involved in its metabolism suggest the possibility of multiple cellular functions for this compound, particularly in tissues displaying high mitotic activity. It has previously been reported that after partial hepatectomy BH₄ levels in the regenerating rat liver are increased concurrently with DNA synthesis. We have reinvestigated this phenomenon with the use of modern techniques for BH₄ analysis, and, in contrast with this earlier report, have found that the BH₄ content and the activities of two enzymes involved in BH₄ metabolism, sepiapterin reductase and quinonoid dihydropteridine reductase, were not altered twenty-four hours after partial hepatectomy. The surgical procedure did, however, produce a vigorous regenerative response as verified by an increase in ornithine decarboxylase activity, and an activation of phenylalanine hydroxylase.

We have also examined the developmental appearance of BH₄ and the first enzyme in BH₄ biosynthesis, guanosine-5'-triphosphate cyclohydrolase, in rat brain and pineal gland with the expectation that these parameters would correlate with specific periods of neuronal proliferation and differentiation. A parallel relationship between BH₄ content and enzyme activity was evident in both tissues during development. In brain, the maximal content of BH₄ and enzyme activity was observed two days prior to, and 10 days after, birth. In contrast, both pineal BH₄ content and enzyme activity became maximal postnatally.

Significance to Biomedical Research and Future Course:

The demonstration that GTP is converted to BH₄ in several different cell and tissue preparations opens up the possibility of studying the regulation of this biosynthetic pathway *in vitro*. Our results already indicate that two different regulatory mechanisms operate in pineal glands and in neuroblastoma cells involving, respectively, inhibition by cyclic-AMP analogs and by exogenous BH₄. Since the rate of synthesis of biogenic amines can be influenced by, among other factors, the concentration of BH₄, insight into the regulation of the biosynthesis of BH₄ should contribute to our understanding of the regulation of synthesis of the catecholamine and indoleamine neurotransmitters.

We plan to continue our studies on these and other mechanisms of regulation of BH₄ synthesis. Publications:

1. Kapatos, G., Kaufman, S., Weller, J., and Klein, D.C. Biosynthesis of biopterin: Adrenergic cyclic adenosine monophosphate-dependent inhibition in the pineal gland. Science **213**, 1129-1131, 1981.
2. Kapatos, G., Kaufman, S., Weller, J., and Klein, D.C. Adrenergic-cyclic AMP regulation of biopterin biosynthesis in the pineal gland. In Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects, Ed. E. Usdin, N. Weiner and M.B. Youdim, Macmillan Publishers Ltd., London, England, pp.231-240, 1981.
3. Kapatos, G., Katoh, S., and Kaufman, S. Identification of 6-lactyl-7,8-dihydropterin (sepiapterin) as an intermediate in biopterin biosynthesis by a cell-free preparation from rat striatum. In Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects, Ed. E. Usdin, N. Weiner, and M.B. Youdim, Macmillan Publishers Ltd., London, England, pp.263-270, 1981.
4. Kapatos, G., Kaufman, S., Weller, J., and Klein, D.C. Development and regulation of rat pineal biopterin content. In Developmental Neurobiology of the Melatonin Rhythm Generating System, Ed. D.C. Klein, Karger AG, Basel, Switzerland, in press.
5. Kapatos, G., Katoh, S., and Kaufman, S. Biopterin biosynthesis by rat brain. J. Neurochemistry in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01081-12 LNP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) The Role of Sensorimotor Cortex in Control of Voluntary Movement | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | E. V. Evarts R. Nelson C. Fromm J. Rajkowski M. Kimura | Chief Staff Fellow Guest Worker Visiting Fellow Visiting Fellow |
| | | LNP LNP LNP LNP LNP |
| | | NIMH NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Neurophysiology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 4.00 | PROFESSIONAL: 4.00 | OTHER: |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project involves utilization of <u>single neuron recording</u> and <u>operant conditioning</u> techniques in behaving <u>monkeys</u> to study <u>brain mechanisms</u> underlying <u>voluntary movement</u> . Monkeys are trained to make precise movements of a handle whose movement controls a visual display, and <u>stimuli</u> are delivered via the handle by means of an electronically controlled <u>torque motor</u> in order to determine how <u>sensory feedback</u> is processed. Using these methods we have shown that (1) motor cortex <u>pyramidal tract neurons</u> (PTNs) exhibit intense modulation during precise small movements involving relatively slight changes in amount of <u>muscle activity</u> . (2) A large proportion of the PTNs in <u>primary motor cortex</u> (MI) are engaged in controlling early-recruited motoneurons during <u>finely-graded movements</u> . (3) MI PTNs are strongly modulated with different <u>loads</u> especially in the vicinity of zero load. (4) There are significant differences between large and small PTNs with respect to their <u>load-frequency relations</u> . | | |

Project Description:

In previous work of this project on motor cortex, primary attention has been focused on a class of motor cortex neurons which send their axons to the spinal cord via the pyramidal tract. These neurons are referred to as pyramidal tract neurons (PTNs) and are of particular importance because they convey the final output from motor cortex to the spinal cord regions from which impulses pass out to the muscles to generate movement. Motor cortex pyramidal tract neurons (PTNs) exhibit intense modulation during precise small movements involving relatively slight changes in amount of muscle activity. This fact has led to the idea that a large proportion of the PTNs in primary motor cortex (MI) may be engaged in controlling early-recruited motoneurons during finely-graded movements. Current work on this project was designed to test this idea through an examination of firing rates of MI PTNs at different loads with special attention to changes of PTN discharge frequency in the vicinity of zero external load. The hypothesis that many PTNs are involved in controlling early-recruited motoneurons implies that there should be marked changes in PTN discharge frequency for small load changes near zero. The results that we have obtained support this hypothesis, and in addition demonstrate that there are significant differences between large and small PTNs with respect to their load-frequency relations.

Major Findings:

PTNs show their greatest frequency changes for small changes of load near zero-load, at a time when relatively few motor units of skeletal muscle are active. For these fine adjustments of force, activity of a large portion of PTNs appears to control relatively few early-recruited alpha-motoneurons, in addition to larger numbers of other target neurons such as gamma-motoneurons. The project has also demonstrated two principal relationships between static force and tonic firing rates of PTNs in MI. One group of PTNs was related to magnitude and direction of load over the entire range of forces examined. A second group of PTNs exhibited graded frequency changes over a limited range of force that differed for each neuron, thus resulting in sets of s-shaped functions. The functional significance of these two principal relationships to muscle force is supported by the significant correlations with the antidromic latency (ADL) of PTNs. PTNs with large axons have rapid axonal conduction velocities (as recognized by short ADLs), whereas small PTNs have lower conduction velocities and longer ADLs. Encoding of force by frequency gradation over the entire range of load is a feature of long-latency, small PTNs. These results leave no doubt that long-latency PTNs have lower recruitment thresholds for tonic firing than short-latency PTNs. Thus, small PTNs maintain relatively higher discharge frequencies in the absence of external load. Finally, results of the project emphasize the similarities between PTNs and fusimotor neurons. Recent studies in man have demonstrated that muscle spindle afferents (and by inference fusimotor neurons) exhibit orderly recruitment with increasing isometric contraction of the receptor-bearing muscle. The conclusion that in isometric contractions motoneurons and fusimotor neurons are influenced in parallel by the descending signals of PTNs is consistent with observations on PTNs.

Interpretations and Conclusions:

Just as there is a "size principle" in spinal cord motoneurons (MNs) whereby smaller MNs tend to be tonically active at low levels of muscular contraction and larger MNs are active phasically during intense exertion, so too there is a relationship between axonal conduction velocity and tonic versus phasic activity in PTNs: smaller PTNs are tonically active even during absence of any discoverable muscular contraction, whereas many larger PTNs are silent in the absence of overt muscular activity exhibiting discharge only when muscles become active. A second major difference between PTNs and MNs is seen in the fact that a very large proportion of motor cortex PTNs related to a given movement exhibit intense modulation for movements that involve activity of a relatively small fraction of the corresponding MNs. Indeed, just as a very large part of the motor cortex is focused on those muscles which are important in precisely controlled movements, it is also the case that a very large proportion of PTNs within an area of cortex controlling a given movement is focused on that fraction of the motoneuron pool which is early recruited and which is of critical importance in precise, fine control. Here then, one sees both parallels and differences between PTNs and MNs. Finally, in contrasting PTNs and MNs one should note a most fundamental difference: a given MN sends its axon to muscle fibers of one and only one muscle, whereas a given PTN sends its axon to motoneuron pools of a number of different muscles as well as to other cortical areas and to subcortical motor control centers. In many ways, the PTN has certain properties resembling those of the "command neuron" with an axon that diverges to the set of elements whose coordinated activity is necessary for a particular movement. This fact has been demonstrated unequivocally by the results of recent investigations in which HRP has been injected intra-axonally into a single pyramidal tract axon allowing the identification of the widespread ramification of its terminals to motoneuron nuclei of a number of different muscles on both sides of the spinal cord.

Proposed Course:

Within the next year we intend to extend these studies to obtain more data on PTNs and non-PTNs in sensorimotor cortex.

Significance to Biomedical Research and to the Program of the Institute:

This project seeks to apply basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in red nucleus, basal ganglia, and cerebellum.

Publications:

Evarts, E.V. and Fromm C.: Transcortical reflexes and servo control of movement. Can. J. Physiol. Pharmacol. 59: 757-775, 1981.

Evarts, E.V.: Control of voluntary movement by the brain. In Matthysse, S. (Ed.): Psychiatry and the Biology of the Human Brain. Elsevier/North Holland, 1981, pp. 139-164.

Evarts, E. V.: Role of motor cortex in voluntary movements in primates. In Brookhart, J. M., Mountcastle, V. B., Brooks, V. B. and Geiger, S. R. (Eds.): Handbook of Physiology, The Nervous System II, Chapter 23. Vol. 11, Motor control, Part 2. Bethesda, Maryland, American Physiological Society, 1981, pp. 1083-1120.

Fromm, C., Evarts, E.V., Kröllner, J., and Shinoda, Y.: Activity of Motor Cortex and Red Nucleus Neurons During Voluntary Movement. In Pompeiano, O. and Ajmone Marsan, C. (Eds.): Brain Mechanisms and Perceptual Awareness. New York, Raven Press, 1981, pp 269-294.

Evarts, E.V.: Haltung und Bewegung/Posture and Movement. Brain activity during voluntary movement: contrasting features of information output from motor cortex, sensory cortex, and red nucleus in the monkey. In Freiburger Universitätsblätter, Hirnforschung Grundlagen und Klinik, presented at the Symposium in honor of Professor Richard Jung, October, 1981, pp. 15-19.

Evarts, E. V.: Cortico-cortical and thalamo-cortical inputs to precentral motor cortex in the monkey. In Orbach, J. (Ed.): Neuropsychology after Lashley. New Jersey, Lawrence Erlbaum Associates Publishers, 1982, pp. 409-417.

Fromm, C., and Evarts, E.V.: Pyramidal tract neurons in somatosensory cortex: central and peripheral inputs during voluntary movement. Brain Res. 238: 186-191, 1982.

Evarts, E. V.: Analogies between central motor programs for speech and for limb movements. In Grillner, S., Lindblom, B., Lubker, J. and Persson, A. (Eds.): Speech Motor Control, Vol. 36. Wenner-Gren Symposium Series. New York, Pergamon Press, 1982, pp. 19-41.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01090-06 LNP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies of Central Nervous System Functional Anatomy | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | Miles Herkenham Sandra Moon Edley Ronald P. Hammer Jr. Candace Pert | Research Psychologist LNP NIMH Staff Fellow LNP NIMH Staff Fellow LNP NIMH Pharmacologist BP NIMH |
| COOPERATING UNITS (if any) The Neuroscience Branch | | |
| LAB/BRANCH Laboratory of Neurophysiology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MANYEARS: 3.25 | PROFESSIONAL: 3.25 | OTHER: |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) A sensitive method for <u>light microscopic localization of brain receptors by in vitro autoradiography</u> was developed previously in this laboratory. By this method we have mapped the locations of opiate receptors in the brains of rats and other vertebrates, including <u>primates</u> . Comparisons of tritiated <u>naloxone</u> binding with tritiated <u>enkephalin</u> binding have reinforced the notion of <u>opiate receptor subtypes</u> . These have been followed <u>ontogenetically</u> and <u>phylogenetically</u> and have been related to the <u>dopamine system</u> in the <u>striatum</u> . The possibility of <u>pharmacological manipulation of receptor distribution</u> is being examined. Applications are being pursued for the study of receptors and <u>biologically active peptides and enzymes</u> in unfixed human brains obtained at autopsy. The unfixed, cryostat-cut tissue is amenable for concurrent study of <u>metabolic and functional mapping by 2-deoxy-D-glucose</u> . For example, we have compared <u>phencyclidine (PCP) receptor localization patterns</u> with patterns of altered brain <u>metabolic activity produced by phencyclidine "anesthesia"</u> . The technique is used also for studies related to <u>sleep, circadian rhythms</u> and <u>drug-induced sedation</u> . | | |

I. Studies of CNS Functional Neuroanatomy. Neurochemical Investigations.

Objectives:

For the past four years, a collaborative effort with Dr. Candace Pert in the Biological Psychiatry Branch of the NIMH has been directed toward localizing the brain sites of action of pharmacologically active drugs and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of anatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and terminal distributions of fiber pathways in any given region. By such analysis we can speculatively identify the specific neuronal systems that contain the relevant transmitter and thereby make predictions about the neurochemical's role in normal brain function. Determinations of the locations and densities of the receptors in relevant brain regions can be used in tests of receptor changes during development, after chronic pharmacological manipulation, or in neuropathological tissues.

Methods Employed:

We have successfully developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue slices. Fresh cryostat-cut brain slices are securely attached to glass slides by a process of thaw-mounting and subsequent drying at cold temperatures. Slides are then incubated in solutions containing radiolabeled ligands. Excess and nonspecifically bound ligand is washed off in cold buffered rinses, and the slides are blown dry. The sections are fixed in hot paraformaldehyde vapors under a vacuum, defatted in xylene and alcohol rinses, dried and then dipped in radioactive-sensitive emulsion for autoradiography. Alternatively, fixed sections can be placed in an x-ray cassette and overlain with LKB tritium-sensitive film. The developed film autoradiogram then can be analyzed by a densitometer for computer-assisted quantification of receptor densities. While emulsion coated sections provide high resolution analysis through the microscope, films can be computer-analyzed for rapid density measures or for color-coded image enhancement.

Major Findings:

The method we developed for in vitro autoradiographic localization of brain receptors is now in press. Several lines of evidence have been pursued, both independently and in collaboration with Dr. Pert's laboratory. Autoradiographic localization of opiate receptor subtypes labeled by the " μ " agonist, dihydromorphine or the " δ " agonist, D-ala-D-leu-enkephalin was part of a major biochemical demonstration of binding kinetics and structure-activity relationships performed on slide-mounted tissue sections. The autoradiography and biochemistry together supported the hypothesis that the subtypes are different conformations of a single, interconvertible, dynamic receptor. In a similar fashion, the phencyclidine (σ opiate) receptor was characterized and visualized. Visualization of opiate receptor ontogeny, both pre- and postnatally, suggested other dynamic aspects of opiate receptors: they appear very early in development and, through the process of timed birth and selective elimination, are sculpted into the adult pattern. Such changes may reflect an important role that receptors play in the establishment of neuronal connections. Opiate receptors undergo

phylogenetic changes as well, as suggested by our finding that the ratio of μ to δ receptor binding increases in parallel with "relatedness to humans" (as indicated by cytochrome C analysis of several vertebrate species). Analysis of μ opiate receptor distribution in rhesus monkey cerebral cortex has revealed several general principles; e.g., opiate receptor density is greatest in limbic and polysensory (association) cortices.

These findings, taken together, suggest a role for opiates in brain function that is much more complex than previously thought and indicate that further analysis of the dynamic aspects of the receptor, after pharmacological or behavioral manipulations, might enhance our understanding of its function. Ultimately, we might hope to determine the role that opiates and related neurochemicals play in human brain function, especially in receptor-mediated mental disorders or neuropathology.

II. Metabolic Correlates of Functional Activity

Objectives:

We are currently involved in developing high-resolution autoradiographic techniques for the cellular localization of metabolic activity at the light microscopic level. Patterns of metabolic activity marked by [^3H]2-deoxyglucose uptake are compared in normal, alert rats and in animals given drugs or prior behavioral experience. Using series of adjacent tissue sections from a single animal, patterns of metabolic activity during drug administration can be correlated with the localization of receptors to which the drug binds.

Similar techniques will be useful in our studies of the metabolic development of functional cell systems which have been morphologically characterized. The use of metabolic markers visible at the cellular level of resolution will permit the study of small populations of neurons in the developing brain. We are particularly interested in hypothalamic systems which show sex differences and those involved in cyclical and rhythmic behaviors.

Methods Employed:

We are developing techniques which will permit us to use [^3H]2-deoxy-D-glucose (2-DG) as a metabolic marker of glucose utilization, visible at the cellular level of resolution. Low resolution was a persistent problem in previous autoradiographic localization studies, which utilized [^{14}C]-labeled 2-DG as a marker. The substitution of [^3H]-labeled 2-DG improves resolution, for the particles of [^3H] are less energetic than those of [^{14}C] and form an image closer to their source.

Visualization of metabolic activity of individual neurons requires fixation of the diffusable 2-DG molecule to its uptake site. This may be accomplished by the fixation of 2-DG in situ using perfusion with a light-to-medium strength paraformaldehyde fixative followed by cryosectioning and mounting. After perfusion, 2-DG and its labeled metabolites are retained in brain tissue. Moreover, labeling of cellular regions is enhanced. Current evidence suggests that some of the

2-DG may be incorporated into intracellular glycogen, which is fixed in place during perfusion and does not diffuse from its cytoplasmic location during later phases of aqueous processing. Alternatively, these sections can be quickly dried and exposed to tritium-sensitive film to retain in place the labeled diffusable 2-DG-6-phosphate, which is the major 2-DG breakdown product.

Autoradiographic localization of brain receptors can be compared in the same animal with manipulation-induced alterations in brain metabolism measured by the 2-DG technique. Alternate sections from an animal previously injected with 2-DG are either processed for 2-DG autoradiography as described or for receptor localization. The latter is accomplished by first removing the diffusable 2-DG in preincubation solutions prior to in vitro receptor binding. In this way, the alteration of brain metabolism by drugs or anesthetics may be correlated with receptor binding in those brain regions affected.

Major Findings:

Analysis of 2-DG uptake in animals given anesthetic doses of ketamine has confirmed that phencyclidine-induced changes in brain metabolism occur primarily in limbic regions. Another striking variation is the relatively greater 2-DG uptake in neocortical columns, many of which are in register with granule cell-poor zones of layer IV in somatic sensory-motor cortex. The columnar patterns of uptake occur in restricted cortical zones innervated by nonspecific thalamic or intracortical fibers. The significance of this relationship of selective cortical afferent innervation and its differential activation during ketamine anesthesia is currently under investigation. Superficial layers of the entorhinal cortex and subiculum also show selective and dramatic increases in 2-DG uptake. Assuming that ketamine's effects on metabolism are initially mediated by its action at the phencyclidine receptor, such increased activity may be correlated with the patterns of tract terminations originating in regions rich in these receptors. These results suggest that phencyclidine influences brain metabolism by selectively affecting the activity within neo- and paleocortical systems.

Significance to Biomedical Research and to the Program of the Institute, and

Proposed Course:

The visualization by autoradiographic techniques of opiate receptor locations throughout the CNS has greatly advanced our appreciation of the richness of opiate functions in normal physiology and has opened a surprising number of doors to the investigation of receptor-mediated brain processes. We have just begun to appreciate how receptors influence and control neuronal development and the establishment of neural connections, the interrelatedness of receptor subtypes and of neurochemically distinct systems (such as dopaminergic and opiate interactions in the striatum), the evolution of receptors as markers of synaptic complexity and the significance of species differences. We are encouraged by preliminary comparisons of drug receptors and the altered metabolic profile (as seen by 2-deoxyglucose autoradiography) produced by the same drugs. These findings indicate a productive future in the research of brain function. Some preliminary data on receptor differences, visualized by autoradiography in genetically "nervous" dogs, may be of clinical importance.

Since these differences appear to be pharmacological, identification of correlates in human mental disorders would encourage us to analyze receptor distributions in the brains of deceased humans with histories of Parkinson's disease, Huntington's chorea and schizophrenia.

Publications:

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01091-05 LNP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Motor Function in Patients with Neuropsychiatric Disorders</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Jerome N. Sanes Others: Edward V. Evarts Karl-Heinz Mauritz Von A. Jennings Andreo Larsen | Staff Fellow Chief Guest Worker Staff Fellow Visiting Fellow | LNP NIMH LNP NIMH LNP NIMH LNP NIMH ETB NINCDS |
| COOPERATING UNITS (if any) Experimental Therapeutics Branch, NINCDS | | |
| LAB/BRANCH Laboratory of Neurophysiology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) The purposes of this project are to examine the contributions of central <u>motor programming</u> and <u>afferent</u> input in control of <u>arm movements</u> in <u>normal</u> subjects and patients with <u>sensori-motor</u> disorders, and to study <u>psychomotor</u> performance of patients with <u>central motor</u> disorders. The first set of experiments records <u>muscle activity</u> and <u>kinematics of limb position</u> while (1) subjects manually match a target display with either a skilled <u>rapid</u> or <u>slow movement</u> with a handle whose displacement controls a visual display or (2) <u>maintain postures</u> when limb position is passively changed. Movement amplitude, presence or absence of visual feedback of position, disturbances of the subject's movements and changes in sensory input are independent variables. <u>Large movements</u> are performed <u>accurately</u> independent of manipulation of the experimental variables but accurate performance of <u>small movements</u> becomes increasingly <u>dependent</u> on the <u>absence of limb disturbances</u> during movement. The second set of studies examined a variety of <u>psychomotor</u> variables from patients with Parkinson's disease. The relationships between movement <u>speed</u> , movement <u>accuracy</u> , target size and movement amplitude were studied to develop sensitive measures of psychomotor performance that <u>correlated</u> with clinically determined <u>fluctuations in drug efficacy</u> . | | |

I. Central and Peripheral Control of Movement in Humans.

Project Description:

The importance of afferent information in the control of limb movements is controversial. Whereas it is clear that afferents exert potent physiological effects on spinal motoneurons and cells in supraspinal structures, it has been suggested that some of these afferents contribute little to the final positioning of a limb. A case in point is the observation that muscle spindle activity does not reflect muscle length during rapid movements of large amplitude, thereby casting doubt on a regulatory role for spindles at the end of movements. In addition, physical disturbances imposed during movements, that likely activate muscle spindles, do not appear to modify final limb positioning. There are, however, other experiments demonstrating the importance of afferent input in a variety of tasks performed by humans. For example, ischemic deafferentation of limbs alters position sense and sense of effort. Furthermore, performance of fine motor tasks, such as reproduction of alphabetic characters is also disrupted by ischemic deafferentation and it is noteworthy that inactivation of the gamma loop in humans impaired the ability to tonically activate motor units, though phasic activation was not impaired. It is the object of the present project to continue examination of the role of peripheral inputs in the control of limb movements and postural control. Both normal volunteers and patients with neurological disorders will be studied during performance of movements of varying sizes and when a maintained posture is disturbed by different peripheral inputs. Different types of limb disturbances will be imposed during the movements. Two general experimental approaches are being pursued. In the first, the psychomotor variables of movement error and movement time are studied in relation to physical disturbances. In the second group of experiments, intra- and extramuscular electromyographic activity is examined when the limb is displaced while subjects perform motor tasks or maintain postures.

Methods:

Human subjects are trained to manipulate a handle that is attached to a servo-controlled torque motor while performing extension-flexion of the wrist, elbow or index finger, or abduction-adduction of the index finger. Displacement of the handle causes movement of an oscilloscope beam that is to be matched by the subject with a second, experimenter controlled, oscilloscope beam.

In one series of experiments, subjects perform tracking movements either as rapidly as possible or as accurately as possible. For each of the three movement sizes (5°, 10° and 20°) subjects are given an adequate number of training trials. Independent variables include (1) continuous loads opposing or assisting movement, (2) brief physical disturbances delivered to the arm before or after initiation of arm movement and (3) initial starting position. An additional procedure is to ischemically deafferent the arm below the elbow by wrapping a blood pressure cuff around the arm and inflating the cuff above systolic pressure for about one-half hour. Patterns of muscle activity and tracking errors are analyzed. For other work, the ability of subjects to maintain postural stability of the hand is evaluated when the sustained peripheral input to the limb is altered. In these experiments, the subject has to maintain posture against different loads, when the muscle tendons are vibrated, when there is co-contraction of the agonist and antagonist muscles acting about a joint, when the muscles are fatigued, and when the limb is ischemically deafferented.

Preliminary Findings:

Motor behavior of normal subjects and cerebellar ataxic patients has been studied. Several findings have emerged:

(1) When subjects move rapidly the muscles acting about a single joint show the typical pattern of an EMG burst in the agonist muscles, a burst in the antagonist muscle and then a second burst in the agonist muscle. Each of these bursts of muscle activity is affected by sustained loads opposing movement, the starting position, the size of the movement, the velocity of the movement, whether a mechanical obstruction is encountered during the movement and whether the limb has normal sensation or if the limb is deafferented.

- (a) Load : When the subjects move against a load the agonist bursts increase in size and the antagonist burst decreases in amplitude. The reverse situation occurs if the load assists the movement. Indeed, the second agonist burst may be absent when loads assist movement.
- (b) Starting Position : If the agonist muscle is relatively long at the beginning of a movement then the burst of muscle activity seen in that muscle during a rapid movement will be smaller than if the muscle was short at the beginning of movement. The reverse situation holds for the antagonist muscle.
- (c) Movement Size : The bursts of activity in the agonist and antagonist muscles are larger for larger movements.
- (d) Movement Velocity : The bursts of activity in the agonist and antagonist muscles are larger for movements of higher velocity. The antagonist muscle activity is increased dramatically when high velocity movements are performed.
- (e) Mechanical obstructions : If the limb is briefly obstructed soon after the movement begins, the first agonist burst is prolonged and the antagonist burst is suppressed until the obstruction is removed. The second agonist burst occurs, as usual, after the appearance of the antagonist burst of muscle activity.
- (f) Ischemic and neurological deafferentation : The pattern of muscle activity seen in the intact limb is preserved in the deafferented limb. However, the amplitude and sometimes the duration of all three bursts of activity is decreased when afferent input is removed with the method of ischemic deafferentation. The timing of the bursts is also changed. The antagonist burst occurs earlier than it would in the intact limb, but the second agonist burst often occurs later than it would in the intact limb. Patients with sensory neuropathy show the typical pattern of EMG activity associated with rapid movements. It could be concluded that afferent information related to changes in length of muscles is not necessary for the appearance of the burst of EMG, but that afferent information, both of a tonic and phasic manner, probably contributes to both the duration and amplitude of the bursts of EMG associated with rapid voluntary movements.

(2) When subjects are required to maintain a constant posture with the hand when there is no load opposing movement, brief high velocity ramp disturbances result in a damped oscillatory response by the hand that ultimately results in the hand returning to nearly the same position that it was in before the disturbance onset. This effect is independent of the size of the ramp disturbance. In a larger investigation the effects of changing afferent input to the arm while displacements were being delivered were studied. Thus, the effects of changing

the pre-load, tendon vibration, muscle fatigue, co-contraction, and ischemic deafferentation upon the hands response to various sized displacements were studied.

An important question was whether the mechanical response of the hand would vary according to the size of the displacement. Across movement sizes the static error, that is the point to which the hand returned after the damped oscillatory response had ceased, was not dramatically affected by any of these procedures. Despite the absence of any effect on the bias error of the hand with respect to movement size, the variability in the postural response was increased for all procedures when the hand was passively moved a small amount. This would indicate that the reproducibility of limb positional restoration is not as accurate for small displacements as they are for large displacements. Nevertheless, the frequency of the oscillatory response, an indirect measure of muscle stiffness, was greatest for the small disturbances; not a terribly surprising finding since it was likely that fewer cross bridges in the muscle were broken for the small displacement therefore allowing the viscous stiffness of the muscle along with the reflex response to compensate for the passive displacement of the hand. Alteration of the pre-load resulted in a tendency for the final position to be shifted in the direction that the load was passively pushing the hand. This was especially true for the small movements.

The postural responses of the hands of patients with cerebellar ataxia and sensory neuropathy are different from normal subjects in that there is a greater variability in the static responses for all sizes of passive displacement. Furthermore, the end point error of patients with sensory neuropathy is extremely sensitive to preloads, such that the limb is pushed in the direction that the load exerts its torque.

Another procedure attempted to determine motor acuity of normal subjects and patients with neurological diseases. These studies evaluated the spatial accuracy of repetitive step movements of various sizes. Both normal subjects and patients with peripheral sensory neuropathy performed large and small movements accurately when visual guidance was available. Movement accuracy deteriorated for both normals and patients when visual guidance was absent, but the patient with sensory neuropathy showed two to four times the error than normal subjects. Furthermore, whereas the error of normal subjects decreased in absolute magnitude when smaller movements were performed, the spatial error exhibited by deafferented patients decreased only a small amount when the smallest movements were performed.

II. Objective Psychomotor Evaluation of Neuropsychiatric Patients

Project Description:

In the last three annual reports we described the technical aspects and the use of a computerized system for obtaining objective, quantitative measures of motor function in patients with neurological disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system has been used to record and subsequently summarize data concerning motor functions in more than 50 patients with Parkinsonism participating in clinical trials of the experimental anti-parkinsonian drugs, lisuride, bromocriptine and pergolide. Patients with

cerebellar disorders and essential tremor have also been studied. To establish normative data for movement variables measured on our system, thirty age-matched normal subjects have also been tested. An additional use of the computer system has been to begin studies of psychomotor performance using the methods devised by experimental psychologists. These studies will be useful to provide objective measures of the organization of motor performance and to assist the clinician in diagnosis and evaluation of patients with sensorimotor disorders.

Major Findings:

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects were observed. Parkinsonian patients undergoing treatment with lisuride, bromocriptine or pergolide were tested on a regular basis throughout the course of the build-up and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date have been permanently stored in computer files and are accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

A more recent application of computer evaluation of psychomotor functions of patients with neuropsychiatric diseases has been to employ techniques devised by experimental psychologists that use measures of reaction and movement time to describe processes that occur before and during the selection and execution of motor acts. Our first effort has been to apply the method of Fitts to evaluate the dependence of movement time in a sequence of altering limb movements on the required accuracy and the distance of the movement. Our first patient population were individuals with parkinsonism. Patients held an electrical stylus that was moved between sets of targets that varied in width from 1-4 mm or 0.5-4 cm. The distance between the targets was 2-8 mm or 4-32 cm for the respective target arrays. Movements by normal subjects were required to be within a strict accuracy criterion, whereas patients were encouraged to move as accurately and as rapidly as possible. Variation of movement amplitude and target width altered MT of both normals and patients with PD. Normals and PD patients had different performance on the smallest targets (widths of 1-4 mm, amplitudes of 2-8 mm) such that PD patients were slower and less accurate than normals for all combinations of target size and distance moved. The performance of PD patients on the larger target arrays was slower and less accurate than normals but there was also a steeper rate of change in MT as movement size increased for the various target widths. The greater slope of MT/cm for PD patients for all target widths was related to substantial increases in MTs for the largest movements. Patients with PD also showed slower MTs when the target was small. These findings demonstrate that changes in movement size and target width will modify MT in PD patients different from that of normal subjects. The primary deficit in MT for PD patients was the failure to accurately execute large amplitude movements and movements to small

targets. Thus, as the index of difficulty increases (independent of the source of the increase) patients with PD move slower than normals. Therefore, equations relating MT, accuracy and movement amplitude differ between normals and patients with PD. Since emphasis was given to rapidity, as well as accuracy, of movement it may be expected that patients with PD would perform reasonably well in tasks of this type if they are given ample time to complete a movement.

Significance to Biomedical Research and to the Program of the Institute:

Additional clarification of how tactile and kinesthetic sensory information is used to control skilled motor activity is essential to the understanding of normal and abnormal motor behavior in humans. It is hoped that these results will contribute to the understanding of how movements are initiated and completed. Finally, these studies will potentially develop standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents. The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term evaluation of patients' progress on medication regimens is particularly suited for objective analysis.

Proposed Course:

Future studies concerned with psychomotor performance will continue to investigate the importance of tactile and kinesthetic signals occurring during movements. Thus properties of movements and muscle activity will be investigated in normal and fatigued muscles, from functionally deafferented limbs, and following vibratory or mechanical disturbances delivered to a limb. A variety of movement types (e.g. large/small) and strategies (e.g. fast/slow) will be studied to determine the movements that depend upon sensory inputs from the periphery for accurate completion. More than two years of experimentation will be required to validate and extend the preliminary findings in providing additional information on the control of limb movements in normal and neurologically diseased humans.

The computer evaluation of movement is an ongoing project to provide objective evaluation of motor function in patients with movement disorders. Presently the neuroevaluation system is being used to continue evaluation of neuropsychiatric patients' movements when the accuracy and movement extent are varied. In addition, a new project that will study reaction and movement time during movement sequences will be initiated. The purpose of this study is to determine, objectively, the deficits in retrieval and execution of motor commands by patients with motor disorders.

Publications:

Lewitt, P. A., Gopinathan, G., Ward, C. D., Sanes, J. N., Dambrosia, J. M., Durso, R., and Calne, D. B.: Lisuride versus bromocriptine in Parkinson's disease: A double blind study. Neurology, 32: 69-72, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01092-04 LNP | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Corticocortical Connections in the Sensorimotor Cortex of the Monkey | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Steven P. Wise</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 15%;">LNP</td> <td style="width: 19%;">NIMH</td> </tr> <tr> <td>Other: Michael Weinrich</td> <td>Surgeon</td> <td>LNP</td> <td>NIMH</td> </tr> <tr> <td>Karl-Heinz Mauritz</td> <td>Visiting Scientist</td> <td>LNP</td> <td>NIMH</td> </tr> </table> | | | PI: Steven P. Wise | Senior Staff Fellow | LNP | NIMH | Other: Michael Weinrich | Surgeon | LNP | NIMH | Karl-Heinz Mauritz | Visiting Scientist | LNP | NIMH |
| PI: Steven P. Wise | Senior Staff Fellow | LNP | NIMH | | | | | | | | | | | |
| Other: Michael Weinrich | Surgeon | LNP | NIMH | | | | | | | | | | | |
| Karl-Heinz Mauritz | Visiting Scientist | LNP | NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neurophysiology | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
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| SUMMARY OF WORK (200 words or less - underline keywords) This project consists of neurophysiological and neuroanatomical investigations of the organization of the <u>somatic sensorimotor cortex</u> and its role in the control of <u>primate motor behavior</u> . One of the central questions concerning the organization of the <u>cerebral cortex</u> is how inputs to a cortical region from cortical and subcortical sources combine to contribute to its output. We have chosen to concentrate on the <u>precentral motor cortex</u> (MI), known to project directly to spinal motor neurons, and those cortical areas in close functional association with MI, the <u>supplementary motor cortex</u> (MII); <u>area 3a</u> (a transitional region between MI and the postcentral somatic sensory cortex), and the <u>premotor cortex</u> (area 6). Two related approaches have been adopted for this project: (1) an analysis of the organization of peripheral inputs to the premotor cortex, MI, MII and area 3a and (2) single unit studies of cortical areas which supply inputs to MI. In the past year, we have concentrated on to premotor cortex and its activity in relation to planned movements and their execution. | | | | | | | | | | | | | | |

Objectives:

The inputs to the precentral motor cortex (MI) and its intrinsic neuronal circuitry determine the output of MI neurons, including those projecting to the spinal cord. We hope to gain an understanding of the afferent inputs to MI cortex and their interaction in producing motor cortex output. The long-term objective of this project is to examine the activity of neurons that project into and out of MI and to contrast the functional significance of corticocortical and corticofugal neurons.

Two more general objectives of this project are (1) an improved understanding of the evolution, organization, and role in the control of voluntary behavior of the entire motor cortex, a region which is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery and (2) a better understanding of the cortical fields involved in the sensory guidance of movements and the linkage between sensory signals and motor behavior.

Methods:

Monkeys have been trained to perform one of four tasks. (1) Monkeys were trained to make dorsiflexion (upward) or plantarflexion (downward) movement of one foot upon receiving a visual cue. A motor coupled to a foot pedal delivered somatic sensory stimuli to the foot. For each unit, its relationship to movements and response to peripheral stimuli were evaluated using correlational and statistical criteria. Neurons in MI and MII were contrasted in this experiment. (2) Monkeys were adapted to having a foot attached to a pedal. A servo-controlled motor moved the foot at one of four angular velocities. Several amplitudes of displacement were applied to the foot. The relationship of each unit to the velocity and amplitude of passive foot displacements was evaluated by examining peak and sustained neuronal firing frequency. Neurons in MI and area 3a were examined in this experiment. (3) A rhesus monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly, was illuminated after a randomly varied delay. This key thereby became the next target. A barely discernable visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. Neurons in the premotor cortex were studied in this experiment. (4) The monkeys were conditioned to align two spots of light on a tangent screen in front of the monkey. One of these spots is controlled by the computer (target), the other by arm movements of the animal (position). He was required to align the spots within a small accuracy "window." After a short period of time the target light jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target light dimmed, at which point he was required to move (flex or extend his forearm) rapidly and accurately to the target position. In one-sixth of the trials, the computer selected a "no-go" situation in which physically identical stimuli signal the animal to make no movement. This experiment was designed to contrast neuronal activity in MI and premotor cortex.

For the first three experiments, extracellular unit activity of neurons in the somatic sensorimotor fields (area 3a, MI, MII, and the premotor cortex) was

monitored, recorded on magnetic tape with an 8-channel tape recorder, and analyzed off-line with PDP-12 and PDP-11 computers. For experiment #4, the single unit activity and behavioral data were collected on-line with a PDP 11/03 computer and analyzed off-line with a PDP-11/34 computer.

The past year has been devoted to conducting experiment #4, writing a report of the results of experiment #3, writing a preliminary report of the results of experiment #4, and designing follow-up experiments. These next experiments, designed to explore the neural correlates of motor planning in premotor cortex and the topographic organization of premotor cortex, are in the developmental phases.

Following the recording procedures, small amounts (5-10 μ Ci) of [3 H]-amino acid are injected into either the premotor cortex, MI or MII. By noting the ultimate distribution of radioactivity in the brain, the sites of termination of neurons in the somatic sensorimotor cortex can be determined by tissue autoradiography.

Experimental Findings:

About 2100 units have been studied in seven monkeys examined in this project to date. Several findings and interpretations have been developed:

1. MII and premotor cortex neurons are virtually unresponsive to peripheral inputs, compared with MI neurons in the same monkeys. This finding is somewhat surprising from a neuroanatomical perspective, since MII receives monosynaptic corticocortical input from most subdivisions of the somatic sensorimotor cortex and premotor cortex has a variety of potential somatosensory inputs from cortical regions. However, the lack of profound somatic sensory responsiveness supports the hypothesis that MII and premotor cortex play a role in centrally generated motor programs rather than movements regulated by peripheral feedback.

2. MI, which is very sensitive to peripheral inputs, can be divided into at least two regions, one rostral and the other caudal, on the basis of the broad submodality type of its peripheral inputs (i.e. skin receptors and non-cutaneous receptors). These results suggest that cutaneous inputs to MI, since they predominate in a discrete (caudal) part of MI, may be more important in cortical motor control than previously believed.

3. Both area 3a and MI neurons receive information concerning both the velocity and position of parts of a limb during conditions of passive displacement. Aspects of this finding are being pursued in project Z01 MH 01093-04 LNP.

4. These findings have enabled us to improve the current understanding of cerebral localization in this part of the cortex, notably the relationship of physiologically defined cortical regions to those defined by anatomical methods. Two of these points are most noteworthy: (1) Microelectrode methods reveal that the boundary between MI and MII corresponds to the boundary between two anatomically defined parts of the agranular neocortex (termed areas 4 and 6 by Brodmann in 1909). The boundary between MI and premotor cortex corresponds not with the boundary between areas 4 and 6 drawn by Brodmann (1909) but rather an analogous boundary of von Bonin and Bailey (1947). (2) Area 3a, the transitional field between the agranular and the highly granular somatic sensorimotor cortex, appears to be, as it was originally defined (in the work of C. Vogt

and O. Vogt, 1919), a discrete cortical field characterized by a thin internal granular layer (layer IV).

5. Our study of premotor cortex has shown that most neurons in that cortical field change activity markedly before the onset of a voluntary movement. Their activity is often specific for the direction of arm movement. These neurons are located within the frontal agranular cortex, corresponding to a part of area 6 as defined by the absence of a large population of giant, layer V pyramidal cells in addition to the lack of an internal granular layer (layer IV). The premotor cortex can also be distinguished from the MI representation by its markedly increased threshold for intracortical microstimulation effects. Further, a substantial population of neurons change their activity in relation to motor set and/or signals which indicate the location of motor targets. Preliminary results of experiment #4 (see above) show that at least one class of premotor cortex units are more clearly related to planned motor activity than the signals which trigger those movements.

Significance to Biomedical Research and to the Program of the Institute:

Though it is often assumed in the neurological and neurobiological literature that different cortical fields within the somatic sensorimotor cortex relay specific information (e.g. sensory signals) between each other via cortico-cortical connections, there is very little neurophysiological evidence which supports this conclusion. Because of the potential significance of corticocortical connections and cortical specializations to the mechanisms of normal and pathological motor behavior, the interrelationships between well-defined cortical fields deserves a detailed examination.

Proposed Course: In order to further examine the function of cortical connections, we must acquire more knowledge about the input-output organization of the cortex and the differential roles of the various fields within the somatic sensorimotor cortex. There is also a need for more reliable methods with which to distinguish the cortical fields from each other, especially in awake behaving animals. This project will be continued and developed in the Laboratory of Neurophysiology with the collaboration of Dr. K.-H. Mauritz.

Publications:

Wise, S. P. and Evarts, E. V.: The role of the cerebral cortex in movement. Trends in Neurosci. 4: 297-300, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01093-04 LNP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Role of Somatic Sensory Inputs in the Cerebral Control of Movements | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | Von Jennings Steven P. Wise | Staff Fellow Senior Staff Fellow |
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| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Neurophysiology | | |
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| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> This project is a study of the role of <u>sensory inputs</u> to the <u>cerebral cortex</u> in the <u>control</u> of <u>motor behavior</u> in <u>primates</u>. The first part of the project consists of a comparison of neural activity in the <u>somatic sensory cortex</u> (SI) and the <u>precentral motor cortex</u> (MI). The second part of the project is a detailed examination of the signals which the peripheral receptors are sending to the cortex during perturbation of voluntary movements. Such information should provide important clues concerning the pathway taken by peripheral inputs to MI and its role in the initiation and control of movement. </p> | | |

Objectives:

Though peripheral inputs have been shown to affect MI neurons during movement, the significance and source of these inputs remains unclear. A possible role of sensory inputs to MI may be to modulate patterns of muscle activity which are characteristic of rapid voluntary movements. The electromyographic (EMG) activity associated with these types of movements has a distinctive "triphasic" pattern: an initial burst in the agonist is followed by a burst of antagonist activity which in turn is followed by a second burst in the agonist. A question of some interest has been the extent to which each part of this muscle activity pattern is generated by central commands or by peripheral feedback occurring during movement. We are investigating this question by determining whether MI neurons display triphasic patterns of activity during rapid voluntary movements and, if so, whether this activity precedes the bursts of muscle activity. The answer to these questions are of significant theoretical importance for the role of sensory feedback to somatic sensorimotor cortex.

The previous experiments in this project addressed these problems by examining neuronal activity in those SI areas which are known to be densely and reciprocally connected to MI. It was found that the activity of many neurons in posterior SI regions showed a striking similarity to MI neuronal activity in terms of their relation to limb position and exerted force. Such a similarity is consistent with the hypothesis that peripheral inputs to MI are relayed through SI. These findings are also consistent with the possibility that some SI neurons receive inputs from MI and are involved in the initiation of movement. We are now attempting to determine whether sensory input to MI and SI cortex reflect the magnitude of the difference between an intended movement and the movement which the limb actually executes after interaction with the environment.

Methods:

Two monkeys were seated in a chair with their arms coupled by a plastic sleeve to a servo-controlled torque motor. One monkey was trained to pronate or supinate its forearm while the other monkey was trained to flex or extend its wrist. In experiment #1, alternating active pronation or supination movements of 20° or isometric contractions were made. The movements were made with or against a steady load which was applied by the motor and were followed by periods of steady maintenance of limb position. Sensory stimuli consisting of ramp displacements of the limb (10°) were delivered during the period between movements.

In experiment #2, the monkey was conditioned to make wrist movements of two different mean velocities in each of two opposite directions. During half the trials, the movement of the limb was halted either shortly after the beginning or before the end of these voluntary movements. The experiment allows the comparison of unit activity during control and perturbed volitional movements of a variety of velocities in both directions.

In experiment #1, unit activity and behavioral data were recorded on magnetic tape and analyzed off-line with a PDP-12 computer. For each unit recorded in experiment #1, analysis focused on the frequency and pattern of discharge at each steady-state position and isometrically generated force.

In experiment #2, unit activity and event marker codes were recorded on-line with a PDP-11/03 computer. For this experiment, now in progress, it has been necessary to develop additional analytical capabilities in the off-line neuro-physiological analysis package of the LNP. Accordingly, William Sheriff, of the Research Services Branch, technical development staff, has modified the off-line program to allow assessment of unit activity following the stoppage of movement of different velocities.

Experimental Findings:

Comparison of SI and MI Neuronal Activity

Analysis of single unit activity recorded for 1612 neurons in two monkeys has, thus far, produced the following similarities and differences between SI and MI.

1. Both SI and MI neurons were organized into zones with predominantly cutaneous or non-cutaneous inputs. The cutaneous zone in MI (MI/c) was located in the bank of the central sulcus, while the cutaneous zone in SI included areas 3b and 1. The non-cutaneous zone in MI was rostral to MI/c (this zone was termed MI/r) and in SI the non-cutaneous zone consisted of areas 3a, and 2.
2. The cutaneous neurons in MI and SI were similar to each other in their lack of sensitivity to maintained force or position or to the direction of active and passive movements. In addition, MI and SI neurons with non-cutaneous inputs were similar to each other, but different from cutaneous units in their sensitivity to maintained force and position and to the direction of active and passive movements.
3. A difference between MI and SI was the presence in SI of neurons with a non-muscle-like relation to force and position: a muscle which is more active when the forearm is supinated is always more active when supinating force is applied to an immovable object. In SI, a substantial proportion of cells are active with supinating force (without movement) became more active with the forearm pronated. The presence of both muscle- and non-muscle-like neurons in SI is consistent with that regions' postulated role in sensory processing. In order to interpret inputs which signal a mixture of position and force information it is necessary to have two inputs which code position and force in different ways. In that way two independent functions of two variables are created. In contrast, the lack of non-muscle-like neurons in MI is consistent with its role in motor control. MI output does not uniquely specify force or position independently. Instead it appears to specify muscle tension, which depends on both force and position.
4. In contrast to their similarity during maintained force and position, many of the non-cutaneous SI and MI neurons showed very different patterns of activity during transitions from one state to another. The activity of most MI neurons resembled the pattern of EMG activity during changes in force and position. For example, a unit with increased activity related to maintained supination would almost always be characterized by a phasic increase in activity during a supination movement. In contrast, some SI neurons showed the opposite pattern, i.e. phasic decreases in activity during movements towards positions associated with tonic activity increases.

During the last year, two full-length reports and accompanying illustrative material have been prepared for submission to neuroscience journals. Two of these reports concern the above mentioned findings.

Significance to Biomedical Research and the Program of the Institute:

Further clarification of how sensory information from the periphery is utilized to initiate and control skilled motor activity is essential to a better understanding of normal and abnormal movements in man. Though much is known about the involvement of motor cortex in sensory control of movements, more information is needed concerning the characteristics of sensory input to MI during normal and perturbed volitional movements.

Proposed Course of Project:

Analysis of the differences between cortical areas in the response of their neurons to sensory stimuli and their activity during active movements will continue. We will next turn our attention to the hypothesis, first proposed by Phillips in 1969, that neurons in motor cortex should receive a neural signal that reflects the error between an intended movement and the movement that actually occurs.

Publications:

None

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|---|---|---|---------------------|------------------|--------------|-----|------|--------|--------------|--------------|-----|------|--|----------------|---------------------|-----|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01094-02 LNP | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Information Processing in the Motor Cortex. | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">John P. Donoghue</td> <td style="width: 20%;">Staff Fellow</td> <td style="width: 10%;">LNP</td> <td style="width: 15%;">NIMH</td> </tr> <tr> <td>Other:</td> <td>Von Jennings</td> <td>Staff Fellow</td> <td>LNP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Steven P. Wise</td> <td>Senior Staff Fellow</td> <td>LNP</td> <td>NIMH</td> </tr> </table> | | | PI: | John P. Donoghue | Staff Fellow | LNP | NIMH | Other: | Von Jennings | Staff Fellow | LNP | NIMH | | Steven P. Wise | Senior Staff Fellow | LNP | NIMH |
| PI: | John P. Donoghue | Staff Fellow | LNP | NIMH | | | | | | | | | | | | | |
| Other: | Von Jennings | Staff Fellow | LNP | NIMH | | | | | | | | | | | | | |
| | Steven P. Wise | Senior Staff Fellow | LNP | NIMH | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | |
| Laboratory of Neurophysiology | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | |
| NIMH, ADAMHA, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | |
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| TOTAL MANYEARS: 1.3 | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this three-part project is to examine the <u>input-output organization</u> of neurons in the <u>motor cortex</u> . Rats have been chosen as the primary experimental animal since they are readily available, easy to condition, and have small, lissencephalic brains. (1) Neuroanatomical and neurophysiological techniques are being employed to characterize the <u>first motor cortex</u> (MI) in rats and determine the connectional relationships of MI with other neural structures. (2) We are planning to record neuronal activity from several classes of <u>projection neurons</u> in the forelimb area of MI cortex in awake, behaving rats. In these experiments, the relationship of the neuronal activity to motor output will be examined for each type of neuron, e.g. <u>corticospinal</u> and <u>corticorubral</u> cells. (3) We are combining <u>intracellular recording</u> with <u>intracellular injection</u> of a tracer, horseradish peroxidase, to visualize the intracortical distribution of neuronal processes belonging to identified projection neurons. | | | | | | | | | | | | | | | | | |

Objectives:

The output of neurons in the somatic sensorimotor cortex is closely linked to motor activity in mammals. These cortical outputs arise after intracortical processing of inputs through a complex array of neurons. The experiments described here are designed to elucidate the role of different cell types in the input-output transformations that occur in first motor (MI) subdivision of somatic sensorimotor cortex.

The first step in these studies is to better define MI. This can be accomplished by employing neurophysiological mapping techniques in conjunction with neuro-anatomical pathway tracing and cytoarchitectonic techniques. The second objective is to examine the role in motor cortical function for each of several classes of projection neurons in MI cortex during a simple forelimb motor task. Previous studies have shown that cells in the superficial layers of MI cortex project mainly within the cortex while cells in the deeper layers project mainly to diencephalic, brainstem, and spinal targets. However, except for pyramidal tract neurons, there is little known about the contribution of different types of cortical projection neurons to cortical output or to the control of motor activity. The third objective is to identify the intracortical connections of projection neurons in order to better understand potential routes of information flow within small modules of neocortex.

Methods:

1. Identification of inputs and outputs of MI cortex. For pathway tracing experiments, axonal tracers (histochemical markers or radioactive amino acids) are injected into neurophysiologically characterized cortical regions. Following appropriate survival times the animals are perfused and the brain processed by standard methods to reveal the distribution of tracer substances in the brain and thereby the connections of the injected cortical regions.
2. Chronic recording. Rats are trained to press a bar and maintain a constant force with their forelimb in order to obtain a water reward. In this task the animal must maintain a force level with its forelimb against a stationary lever for a given period of time. After learning the task, a recording chamber is placed over the forelimb region of MI cortex and stimulating electrodes are placed: (a) in the locus coeruleus or midbrain raphe nuclei to stimulate these cortical afferent pathways or (b) in the corticospinal tract, basilar pontine nuclei, red nucleus, thalamus or the contralateral cortex, in order to test for antidromic activation of the MI neurons that project to these structures. Subsequently, single unit recordings are made during task performance. Unit activity, force, and occurrence of bar pressing are recorded on-line with a PDP 11/03 computer and these data are analyzed off-line.
3. Intracortical circuitry The intracortical connections of neurons in MI cortex are identified by intracellular recording and subsequent labeling of single neurons with horseradish peroxidase (HRP) in anesthetized animals. Intracellular recordings are made with glass electrodes filled with 4% HRP in tris/KCl buffer. The distant axonal connections of each neuron are determined with antidromic activation methods.

Stimulating electrodes are placed in the pyramidal tract, corticospinal tract, contralateral cortex, and in some cases, in other subcortical targets of MI neurons such as the red nucleus. Each antidromically identified cell is injected with HRP. At the termination of the experiment, the animal is perfused, the tissue is sectioned with a vibratome, and the distribution of tracer within the cell is demonstrated with standard histochemical techniques.

Experimental Findings.

1. Pathway tracing experiments. Using intracortical microstimulation and axonal transport methods, we have shown that the motor cortex of the rat coincides with a distinct cytoarchitectonic area, the lateral agranular field (AG_1), and also includes part of the adjacent granular cortex of the first somatic sensory area. We have now examined the inputs to AG_1 with axonal transport methods. We have found that AG_1 receives input from ipsilateral and contralateral cortex and several subcortical sites. Ipsilateral cortical projections to AG_1 arise from the first somatic sensory and second somatic sensory areas as well as the frontal agranular cortex that lies rostral to AG_1 . Contralateral input arises from AG_1 . Thalamic input to AG_1 originates from the ventrolateral complex, ventromedial nucleus, central lateral intralaminar nucleus, and the posterior nuclear complex. We are currently examining the outputs of MI cortex in rats that have received injections of tritiated amino acids in that cortical field.

2. Chronic recording experiments. We have established a reliable method for single unit recording in behaving rats. About 100 units have been recorded in somatic sensorimotor cortex during the forelimb task described above. We have observed many units related to force production, and they may begin this activity 100 ms prior to force change. These findings suggest that neurons in rat MI are important in the elaboration and execution of central motor programs. These same areas receive somatic sensory inputs. Thus, MI output can be modulated by sensory feedback during movement. In two animals, the locus coeruleus was stimulated during the bar pressing task. Preliminary data from these animals suggest that the locus coeruleus input enhances the activity-related discharge of some single units in AG_1 during movement.

3. Intracellular experiments. Four pyramidal tract neurons and two commissural neurons have been labeled by intracellular injections of HRP. Preliminary examination of the axonal arborizations of these cells reveals that both types of MI projection neurons have extensive intracortical connections, suggesting that they have an important role in information processing within MI as well as in sending signals to their projection targets.

Significance to Biomedical Research and to the Program of the Institute.

Elucidation of the mechanisms of cortical information processing in the motor cortex, especially the role of different cell types in affecting cortical output, will provide a better basis for understanding normal and abnormal motor and sensory function in man.

Proposed Course of the Project:

This project is now in its initial phases. The majority of effort is now being devoted to the second and third parts of the project: chronic single-unit recording from awake behaving rats and intracellular injections of identified projection neurons. These experiments will be developed during the next year.

Publications:

None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01335-12 SMRA |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies of Schizophrenia | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R.J. Wyatt, Chief, Adult Psychiatry Branch, SMR; J.C. Gillin, Deputy Chief, Adult Psychiatry Branch, SMR; L.B. Bigelow, Clinical Director, WAW Division; R. Wagner, D. Shore, L. DeLisi, S. Zalcman, J.E. Kleinman, D. Weinberger, J. Morihisa, C.N. Karson, N.R. Cutler, S. Potkin, K. Berman, Staff Psychiatrists, Adult Psychiatry Branch, SMR; A. Church, G.N. Ko, C. Kaufmann, W. Lawson, W.J. Freed, Staff Fellows, Adult Psychiatry Branch, SMR; E. Korpi, Visiting Scientist, Adult Psychiatry Branch, SMR; F. Karoum, Chemist, Adult Psychiatry Branch, SMR; H.E. Spoor, Psychologist, Adult Psychiatry Branch, SMR. OTHER: D. Murphy, Clinical Neuropsychopharmacology, NIMH N. Neff, Pharmacologist, SMRP D. Reiss, George Washington University M. Buchsbaum, Biological Psychiatry, NIH I. Hanbauber, National Institute of Health | | |
| COOPERATING UNITS (if any) Laboratory of Neuropsychopharmacology, NIMH; Laboratory of Preclinical Pharmacology, NIMH; Stanford University; George Washington University | | |
| LAB/BRANCH Adult Psychiatry Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 20 | PROFESSIONAL: 16 | OTHER: 4 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The following report summarizes the extensive studies of schizophrenia performed by the Adult Psychiatry Branch during the reporting year 1981-1982. All studies are briefly summarized under subheadings within the appropriate section. | | |

Project Description:

Objectives:

I. Assessment

The objective of our research into the schizophrenias is to investigate etiologies, biochemical processes, neuroanatomical components and treatment. Our work in these areas attempts to produce new knowledge while synthesizing prior information in the psychological, physiological, neurochemical and biochemical disciplines.

A. Computerizing Nursing Data

During the last reporting year the psychophysiology group developed a computer program to allow online entry of nursing behavioral data for the three inpatient units. The program developed enables the rater to respond to serially presented questions regarding each patient. The program also has internal monitors designed to flag inconsistent or improper entries and represent the item to the rater. This program has also been designed with sufficient flexibility to permit the storage over time of data on all patients in the Division so as to permit rapid correlations of behavioral data with other entered data such as physiological or biochemical. The formatting and programming was done using actual raters as test subjects in order to discover, to the extent possible, any possible ambiguity or places where alterations might be necessary. Outside consultants observing the finished product have been uniformly impressed.

B. Premorbid Adjustment Scale (PAS)

Schizophrenia markedly disrupts an individual's psychosocial functioning. Assessment of psychosocial functioning has become a useful area of investigation but is confounded by the schizophrenic process itself. Therefore, considerable research has focused on assessment of the individual's psychosocial functioning prior to the onset of the schizophrenic illness, i.e., the premorbid period. Separating early morbid functioning from premorbid functioning can be difficult. To the degree that this separation can be made, one can relate factors preceeding the disorder to aspects of the disorder itself, such as course, degree of response to treatment, and current symptoms. Do patients with good premorbid psychosocial functioning have better outcomes or require less medication? Are different etiologies suggested by different patterns of premorbid adjustment? Thoughtful planning of therapeutic goals for psychotic patients require understanding and assessing what the individual was like prior to the illness, and to what degree psychosocial functioning and development tasks were mastered prior to becoming ill.

Successful psychosocial functioning is comprised of many components and is difficult to conceptualize and to define, especially in the form of a rating instrument. Further, it has not always been clear what aspects of premorbid life are most characteristic and have most predictive value in a rating scale. The most well-studied premorbid scales have shown poor premorbid adjustment to be related to various parameters including outcome of therapy, duration of hospitalization, and types of symptoms. Most of these scales, however, were developed many years ago. Consequently, the items in many, while calling for subjective ratings, contain anchor points which no longer reflect cultural norms. Further, most scales fail to systematically evaluate premorbid functioning at several periods of life.

In the summer of 1977, the Center for the Studies of Schizophrenia held a workshop focusing on difficulties in assessing premorbid adjustment. The present scale was developed in response to suggestions and apparent consensus that came out of the workshop. We

wished to develop a scale that was useful for research purposes and 1) that conceptualized premorbid adjustment in terms of the attainment of certain developmental goals...achievement of the developmental goals was viewed as necessary milestones for healthy functioning; and 2) that considered attainment of these goals as specific age-related tasks. Thus, the individual with a poor premorbid adjustment was viewed as one who might not achieve one or more of these developmental goals prior to the onset of his illness, or who might achieve them at a later period in his or her life than is considered appropriate. Premorbid "competence", in social terms, could thus be measured by the extent to which the individual was able to meet expectations appropriate to his or her age and sex prior to becoming ill.

The PAS (Premorbid Adjustment Scale) is a rating scale designed to evaluate at each of several periods of the subject's life, the level of functioning in four major areas: social accessibility-isolation, peer relationships, ability to function outside the nuclear family, and capacity to form intimate socio-sexualities. Items evaluating age appropriate functioning in these areas are repeated for each period of the subject's life. The four life period sections are as follows: Childhood, up to 11 years; Early Adolescence, 12-15 years; Late Adolescence, 16-18 years; and Adulthood, 19 years and beyond. The final section, labeled General, is more global, containing items meant to estimate the best level of functioning that the subject achieved in his or her lifetime prior to becoming ill, as well as the time span and characteristics of onset of illness, and general information such as amount of education. Scale items are made up of a combination of original, adopted, and modified items from the Phillips, Gittelman-Klein, and Elgin scales. The adopted items were chosen from these scales based on their suitability to each time period of the subject's life, and their suitability for estimating the successes and failures in the subject's development.

The scale is intended to measure only "premorbid" functioning, with "premorbid" being defined as the period ending six months prior to first psychiatric hospital admission or psychiatric contact, or six months prior to evidence of characteristic florid psychotic symptomatology including delusions, hallucinations, thought disorder, inappropriate or bizarre behavior or gross psychomotor behavior where the symptoms are not apparently due to organic causes. Only those life periods that are premorbid by this definition should be rated on the scale, regardless of the present age of the subject; e.g., a 39-year-old patient who had his first psychotic break at age 17 would not be rated in the adult section (age 19 and beyond), but should be rated on all the other scales, including the General section.

Ratings are based on histories derived from the subject's hospital records or family members. When it is felt that the patient is reliable, a personal interview may also be carried out to complete the ratings.

Rating

Each section of the scale contains a number of items with a scoring range of 0-6. The "0" end of the continuum denotes the hypothetically healthiest end of the adjustment range, and the "6" the hypothetically least healthy end. Descriptive phrases serve as rough anchor-points. The rater selects the number that corresponds most closely to the description phrase nearest it. Not every aspect included in a descriptive phrase is necessary for the rating. For example, "poor adaptation, dislike school, frequent truancy and frequent discipline problem" all appear opposite a rating of "4" on the school adaptation item. A child who has a poor adaptation to school, dislikes it, and who is a discipline problem may be rated a "4", even though that child does not have a history of truancy. When the rater does not have sufficient information regarding a particular item, that item is not scored.

Scoring

The ratings received for each item in a section are summed and expressed as total score divided by the possible score. The possible score indicates the highest score obtainable by adding the maximum score for all items completed. Thus, if a subject receives ratings of 2,3,3 and 2 for the four items in the childhood section, the total score for that section is 10. The possible score is 24 ($6+6+6+6$), and the total score divided by the possible score is .42. When no information is available for a particular item, the item is not scored. The score for the section then is expressed as total score/possible score for the items rated. For example, if only three out of four of the items in the preceding example are scored, possible scores become 18 ($6+6+6$) instead of 24. If the patient received the same ratings as in the previous example, except for one unratable last item, the total section score would be 8 ($2+3+3$). In this case, total score/possible score is $8/18$ or .44.

An overall score for the whole scale may be calculated by averaging the subscale scores for all the subscales rated for the patient. An average is preferred to a total score in order to avoid bias that would occur in cases in which the sum of a few highly scored subscales would result in the same score as the sum of several moderately or low-scored subscales, when age of onset of illness or lack of information leads to some subscales being left out.

Reliability

Inter-rater reliability was determined in two studies. In the first study, two raters familiar with and experienced in the use of the PAS rated 11 patients. Both raters reviewed the patients' charts for psychosocial histories. In some instances, patient interviews were conducted with both raters present. After chart reviews and patient interviews were completed, the raters independently completed the PAS for each patient. The intraclass correlation coefficient for the two raters was $r=.85$, $p=.0001$.

In the second study, inter-rater reliability among raters from another hospital who were unfamiliar with the scale and untrained in its use was investigated. Three raters at a California Veteran's Administration Hospital independently rated patients from chart histories alone. When these ratings were completed, the chart histories were reviewed by the two experienced raters. The intraclass correlation coefficients for the three Veteran's Administration Hospital raters was $r=.40$, $p=.01$ and was $r=.85$, $p=.0001$ for the NIMH raters. The correlation for all five raters was $.74$, $p=.0001$.

Validity

Comparison with a normal population. A group of 76 normal controls (10 females and 66 males) made up of students, Air Force enlisted personnel, and employees of Saint Elizabeths Hospital, were rated on the PAS. Control subjects were told that the rating scale was being used to compare social development in "normal" persons with that of persons who had become mentally ill. The control subjects were interviewed by one of the two raters who were familiar with the scale. The raters then filled out the PAS for the control subjects based on the information gathered in the interview. The means \pm standard error of the mean for each subscale and the average score for the normals and for a group of 86 schizophrenic patients (12 female and 74 male) are shown in Table 1. The patient population was from Saint Elizabeths Hospital and had volunteered to be a part of the study. The normals were

significantly different ($p < .01$, two-tailed t-test) on every subscale and on Average score from the patient population.

Table 1

MEANS (+ SEM) FOR NORMAL CONTROLS AND CHRONIC SCHIZOPHRENIC PATIENTS ON EACH SUBSCALE OF THE PAS AND AVERAGE SCALE SCORE

| Normal | Childhood | | Early Adolescence | | Late Adolescence | | Adult | | General Average | |
|----------------------------|-------------|-----|-------------------|-----|------------------|-----|-------------|-----|-----------------|-------------|
| | Mean | P | Mean | P | Mean | P | Mean | P | Mean | P |
| Normal Controls (N) | .23 (76) | .01 | .21 (76) | .01 | .17 (76) | .01 | .12 (76) | .01 | .09 (76) | .16 (76) |
| Schizophrenic Patients (N) | .35 (77) | .02 | .44 (76) | .02 | .52 (61) | .02 | .31 (32) | .02 | .51 (86) | .45 (86) |

Out-patients versus chronically hospitalized. Patients were drawn from wards at Saint Elizabeths Hospital or were outpatients from Saint Elizabeths. PAS ratings for the schizophrenic patients were accomplished by a combination of chart history review and personal interviews by the same raters who rated the normal subjects. All patients' charts were reviewed by the same raters who rated the normal subjects. All patients' charts were reviewed and interviews were conducted with patients who were well enough to cooperate. The majority of the patients were drawn from research wards, where care had been taken by the ward physicians and the psychiatrist staff to acquire as full and detailed a history as possible for research purposes. Of the total group of 86 schizophrenic patients rated on the PAS, 19 were outpatients at the time the ratings were done. Approximately half of the remaining patients had been continuously hospitalized for seven years or more. When we compared premorbid adjustment of the patients who were currently outpatients to that of the patients who had been continuously hospitalized for at least seven years, the PAS successfully discriminated between the two groups. As expected, the patients who had been continuously hospitalized had significantly worse premorbid adjustment scores. Significant discrimination was obtained for all subscales except Childhood. (Early Adolescence, $p=.02$, Late Adolescence, $p=.009$, Adulthood, $p=.001$, General, $p=.02$ and Average scores, $p=.002$. One way ANOVA, see Table 2).

Table 2

ONE-WAY ANALYSIS OF VARIANCE, MEAN + STANDARD ERROR OF THE MEAN (SEM) AND (N) FOR OUTPATIENTS AND INPATIENTS ON THE SUBSCALES OF THE PAS

| | <u>Outpatients</u> | <u>Inpatients</u> | <u>F</u> | <u>P</u> |
|-----------|--------------------|-------------------|----------|----------|
| Childhood | .37 + .03 (17) | .45 + .05 (19) | 1.57 | .22 |

| | | | | |
|-------------------|-------------------|-------------------|------|------|
| Early Adolescence | .40 + .04 (19) | .55 + .05 (16) | 5.98 | .02 |
| Late Adolescence | .46 + .05 (18) | .67 + .05 (11) | 8.0 | .009 |
| Adulthood | .34 + .05 (9) | .71 + .08 (4) | 18.1 | .001 |
| General | .44 + .05 (20) | .59 + .04 (20) | 6.05 | .02 |

Length of hospitalization. Reliable information regarding length of hospitalization was available for 39 patients. Average premorbid adjustment scores for these patients were correlated with the number of months of hospitalization (Pearson $r=.41$, $p=.006$). Chronic hospitalization was related to poor premorbid adjustment. When the individual subscale scores of the PAS were correlated with length of hospitalization, the Childhood ($r=.47$, $p=.003$), Early Adolescence ($r=.38$, $p=.03$), and General ($r=.36$, $p=.03$) subscales and Average scale ($r=.46$, $p=.005$) were significantly correlated. See Table 3.

Table 3

LENGTH OF HOSPITALIZATION AND PREMORBID ADJUSTMENT

| <u>Subscale</u> | <u>Pearson "r"</u> | <u>P</u> |
|-------------------|--------------------|----------|
| Childhood | .47 | .003 |
| Early Adolescence | .38 | .03 |
| Late Adolescence | .28 | .13 |
| Adulthood | .56 | .12 |
| General | .36 | .03 |
| Average | .46 | .005 |

For 40 patients the exact age of onset could be determined. These patients were divided by age of onset into four groups: age of onset of 15 and below (eight patients, mean + SEM months hospitalization 174 ± 54.3 months), 16 to 18 (18 patients, 109.7 ± 83.6 months), 19 to 24 (13 patients, 133.3 ± 46.3 months) and over 25 (one patient, 216 months). The ANOVA between the groups for length of hospitalization and age of onset was insignificant ($F=.60$, $p=.62$). See Table 4. Table 5 provides item by item correlations for length of hospitalization.

Table 4

ONE-WAY ANALYSIS OF VARIANCE BY AGE-OF-ONSET OF SUBSCALES, AVERAGE SCORE
AND LENGTH OF HOSPITALIZATION; MEANS \pm SEM AND (N)

| | <u>15 or less</u> | <u>16-18</u> | <u>19-24</u> | <u>Over 25</u> | <u>F</u> | <u>P</u> |
|------------------------------|-------------------------|--------------------------|--------------------------|-----------------------|----------|----------|
| Childhood | .55 \pm .01 (9) | .37 \pm .04 (26) | .40 \pm .04 (22) | .29 \pm .06 (9) | 2.75 | .05 |
| Early Adolescence | .54 \pm .06 (4) | .46 \pm .04 (27) | .44 \pm .04 (24) | .36 \pm .06 (10) | .99 | .40 |
| Late Adolescence | ----- | .54 \pm .06 (16) | .54 \pm .05 (25) | .42 \pm .07 (10) | 1.18 | .32 |
| Adulthood | ----- | ----- | .51 \pm .07 (14) | .35 \pm .05 (11) | 3.53 | .07 |
| General | .70 \pm .05 (9) | .46 \pm .04 (27) | .49 \pm .04 (26) | .36 \pm .04 (12) | 5.5 | .002 |
| Average | .63 \pm .07 (9) | .45 \pm .04 (27) | .46 \pm .03 (26) | .35 \pm .04 (12) | 4.43 | .007 |
| Length of Hospitalization | 174.9 \pm 54.3 (8) | 109.7 \pm 19.7 (18) | 133.5 \pm 46.3 (13) | 216 (1) | 0.60 | .62 |

Table 5

CORRELATIONS OF INDIVIDUAL ITEMS OF THE PAS
WITH LENGTH OF HOSPITALIZATION

| | <u>Length of Hospitalization</u> | |
|-----|----------------------------------|----------|
| | <u>r</u> | <u>p</u> |
| C1 | .39 | .02 |
| C2 | .48 | .004 |
| C3 | .51 | .004 |
| C4 | .40 | .02 |
| EA1 | .36 | .05 |
| EA2 | .52 | .004 |
| EA3 | .41 | .02 |
| EA4 | .32 | .07 |
| EA5 | .33 | .08 |
| LA1 | .24 | .21 |
| LA2 | .22 | .23 |

| | | |
|-----|-----|------|
| LA3 | .50 | .008 |
| LA4 | .24 | .21 |
| LA5 | .19 | .33 |
| A1 | .67 | .10 |
| A2 | .60 | .11 |
| A3 | .51 | .16 |
| G1 | .39 | .02 |
| G2 | .15 | .38 |
| G4 | .15 | .40 |
| G5 | .05 | -.80 |
| G6 | .29 | .09 |
| G7 | .38 | .03 |
| G8 | .42 | .02 |
| G9 | .44 | .01 |

Insidious versus acute onset. Item number three in the General subscale assesses rapidity of illness onset by change in work or school performance. Based on patients' scores on this item, we compared premorbid adjustment in patients who had an acute onset (defined as less than three months) to that of patients whose onset was rated as insidious (for whom it was rated difficult or impossible to determine the onset of deterioration). Twelve patients had been rated as having an acute onset of less than three months and 10 an insidious onset. The scale successfully discriminated between the two groups of patients on Average PAS and for all but the Adult subscale (Childhood, $p=.004$, Early Adolescence, $p=.005$, Late Adolescence, $p=.005$, General, $p=.0001$, Average, $p=.0001$, one way ANOVA). See Table 6. Again, insidious onset was related to poor premorbid adjustment, while patients with an acute onset tended to have better premorbid adjustment.

Table 6

ONE-WAY ANALYSIS OF VARIANCE OF TYPE OF ONSET OF ILLNESS AND PAS SUBSCALES, AVERAGE SCORE, AND PHILLIPS SCORE; MEANS \pm SEM AND (N)

| | <u>Insidious</u> | <u>Acute</u> | |
|-------------------|------------------|---------------|-------|
| PAS | 29 \pm .05 | 60 \pm .09 | .004 |
| Childhood | (11) | (9) | |
| PAS | .31 \pm .06 | .63 \pm .08 | .005 |
| Early Adolescence | (12) | (8) | |
| PAS | .35 \pm .07 | .70 \pm .07 | .005 |
| Late Adolescence | (9) | (6) | |
| PAS | .27 \pm .04 | .57 \pm .17 | .06 |
| Adult | (5) | (3) | |
| PAS | .29 \pm .05 | .74 \pm .05 | .0001 |
| General | (13) | (10) | |
| PAS | .30 \pm .05 | .69 \pm .06 | .0001 |
| Average | (13) | (10) | |

| | | | |
|----------|--------------------|-----------------|-----|
| Phillips | 22.9 + 1.8 (12) | 16 + 3.0 (7) | .07 |
|----------|--------------------|-----------------|-----|

Prediction of subdiagnosis. Premorbid adjustment as measured by the PAS has been shown to be reasonably predictive of type of onset of illness, length of hospitalization, and need for continuous hospitalization for both the subscales and the Average score. The scale was less successful in predicting outcome for schizophrenic subcategories. Following confirmation of a schizophrenic diagnosis, the patients were further categorized as either chronic undifferentiated, paranoid, or chronic undifferentiated with paranoid features as determined by historical review, semi-structured interview, and staff consensus on DSM II categories. A one way ANOVA based on these subdiagnoses was significant for the diagnosis factor on the Late Adolescence ($p=.05$), and Adult ($p=.01$) subscales. Average scores indicated a trend in tendency towards the same direction ($p=.056$). A Newman-Keuls test indicated that the difference between the paranoid and the paranoid features groups accounted for the significant discrimination in these subscales. The paranoid subgroup had the best premorbid adjustment of the three schizophrenic subgroups.

Relationship to other measures of abnormality. Fifty-one of the chronic schizophrenic patients rated on the PAS also had computerized tomography scans (CT scans) done as part of a study that looked at brain abnormalities in chronic schizophrenia. Twenty-one patients had abnormal scans consisting primarily of enlarged lateral ventricles. Cortical atrophy was observed in three patients and atrophy of the anterior cerebellar vermis in two. The premorbid adjustment scores were significantly worse in the patients with abnormal CT scans compared with patients having normal scans, as measured on the Childhood ($p<.03$) and Early Adolescence ($p<.02$) subscales. Further, all patients who had severe maladjustment assessed by the Childhood subscale (rating over .50) had abnormal scans compared to three of 13 with normal scans (Fischer Exact Probability, $p<.0001$). None were diagnosed as childhood schizophrenics.

Comparison of the PAS and the Phillips Scale. One of the most widely used premorbid adjustment scales is the Premorbid History section of the Phillips Prognostic Rating Scale. Twenty patients rated on the PAS were also rated on the Premorbid History part of the Phillips scale, in order to compare the PAS with the most standard scale currently in use. Age of onset inversely correlated significantly with Early Adolescence, Late Adolescence, General and Average PAS scores. The correlation between the age of onset and the Phillips score ($r=.31$, $p=.21$) is not significant. Correlations calculated for only those patients who were also rated on the Phillips scale are, in most instances, lower and do not reach significance, as might be expected with a small "n". However, correlations between length of hospitalization and Childhood, Adulthood, and General subscale scores and Average score on the smaller PAS population remain higher than the correlation between the Phillips score and length of hospitalization, and approach significance on the Childhood subscale. The PAS did about as well as the Phillips in the case of correlations between age of onset and premorbid ratings in the subpopulation.

Information on type of onset of illness (insidious or acute) was available for 19 of the 20 rated on the Phillips scale. The mean Phillips scores for patients with insidious versus acute onset of illness were 22.7 ± 1.8 and 16 ± 3.0 respectively. The difference was not significant. The PAS differences in type of onset are significant (Table 6).

Typically, a rating of "3" on the Phillips scale has been taken as the dividing point for predicting good or poor outcome. Using this criterion, patients for whom CAT scan information was available were divided into "poor" and "good" premorbid groups and "normal"

or "abnormal" CAT scan groups. A Fischer Exact Probability statistic was computed and was not significant ($p=.77$), indicating that premorbid adjustment as measured by the Phillips and dichotomized in this way does not differentiate between patients with "normal" and "abnormal" CAT scans as does the PAS.

The Premorbid Adjustment Scale (PAS) was devised primarily to measure the degree of success in attainment of certain developmental goals at each phase of a subject's life. Social isolation was felt to be one of the clearest signs of poor premorbid adjustment, particularly if present in Late Adolescence. The capacity to make intimate sexual attachments with others and to function successfully away from home (in school, for instance), were felt to be vital. Items dealing with these aspects of premorbid functioning were thus assessed in each life period by the appropriate subscale. Emphasis was placed largely on asocial characteristics of social functioning; however, some items in the scale (the school adjustment items) tap antisocial behavior as well. It has been suggested that asocial premorbid adjustment may characterize a different type of outcome than does anti-social premorbid adjustment, that is, that individuals who act out against society develop different kinds of mental problems than do people who withdraw from society. The PAS in its present form cannot discriminate between these different types of individuals.

More data are needed and are currently being collected regarding the influence, if any, on scale scores of gender, socio-economic level and race. We are also studying premorbid adjustment in acute schizophrenic patients.

C. Neurological "Soft Signs"

Multiple neurological abnormalities are seen in some schizophrenic patients. These abnormalities often correlate with one another; for example enlarged ventricular brain ratios correlate with impaired performance on the Halstead-Reitan Neuropsychiatric Battery. In this same population, at a percentage sometimes reaching 30%, these neurologic "soft-signs" suggest organic impairment. Previous studies have not clarified the relationship of neuroleptic medications to these "soft-signs" nor compared the prevalence of these "soft-signs" to a controlled population.

To investigate this relationship we have been examining patients on and off neuroleptic medication (so these patients can act as their own controls) and comparing them to age and gender matched controls selected from laboratory personnel. Data are being collected at this time.

II. Biochemistry: Human and Animal Studies

A. Human: Peripheral Measures

1. Lymphocyte Monoamine Oxidase

Monoamine oxidase, the enzyme responsible for the oxidative deamination of several biogenic amines, is found in human brain in at least two forms, type A and type B. Substrates for type B monoamine oxidase include dopamine and phenylethylamine, two compounds hypothesized to be associated with schizophrenia. Human platelets and lymphocytes both contain type B.

As a preliminary step in our examination of lymphocyte function in schizophrenic patients, we have studied lymphocyte monoamine oxidase. A group of 62 chronic schizophrenic patients and 113 controls had their blood drawn for determination of lymphocyte monoamine oxidase activity. An additional 23 available first-degree relatives of the schizophrenic patients were included in the study.

Blood samples were collected for preparation of lymphocytes. Lymphocytes were separated from whole blood and centrifuged for 50 minutes. The lymphocyte-containing band was removed, washed twice with isotonic saline, and the pellet stored at -70°C until assayed. Pellets were resuspended and sonicated for 30 seconds. Monoamine oxidase activity was determined by using benzylamine HCl as the substrate at a final concentration of 5.6 mM in 0.067 phosphate buffer. The benzylamine concentration was above that determined to give maximal enzyme activity. Product concentration was measured at two hours, during which time the reaction was found to be linear. Monoamine oxidase activity is expressed as nanomoles of benzylamine utilized per two hours per milligram of protein. Split samples prepared and assayed in this way had a same-day coefficient of variation of 10.3 and a separate-day variation of 12.3. All monoamine oxidase assays were performed by a biochemist blind to the diagnoses of the patients. Patients were diagnosed and subtyped without knowledge of their monoamine oxidase activities.

A subgroup of 10 patients had their lymphocyte monoamine oxidase levels determined when they were unmedicated, as well as medicated, to ascertain any effect of antipsychotic medication on monoamine oxidase activity. A total of 16 of the 62 patients had monoamine oxidase activity determined while they were drug-free. Patients were subgrouped for analysis by gender, age, race, medication, and clinical subdiagnosis. All statistics were two-tailed t-tests unless otherwise stated.

The 62 chronic schizophrenic patients had a significantly lower mean \pm standard deviation lymphocyte monoamine oxidase activity than the 113 controls. First-degree relatives had a mean activity midway between both groups.

There was no significant difference in lymphocyte monoamine oxidase activity between males and females in either the schizophrenic or control groups, although in both groups a small trend existed for females to have a higher mean than males (Table 1). Both male and female patient groups had significantly lower lymphocyte monoamine oxidase than their corresponding control groups.

Table 1

MEAN LYMPHOCYTE MAO ACTIVITY¹

| | <u>Patients</u> | | | <u>Controls</u> | | | <u>p value</u> |
|-------------|-----------------|-------|----|-----------------|-------|-----|----------------|
| | Mean | SD | n | Mean | SD | n | |
| Total group | 15.09 | 11.6 | 62 | 26.78 | 16.09 | 113 | 0.001 |
| Males | 14.37 | 10.48 | 43 | 26.65 | 16.43 | 88 | 0.001 |
| Females | 16.7 | 14.0 | 19 | 27.82 | 15.37 | 25 | 0.02 |
| Ages 18-29 | 14.43 | 11.6 | 29 | 26.78 | 17.16 | 71 | 0.001 |

| | | | | | | | |
|------------------------|-------|-------|----|-------|-------|----|--------------------|
| 30-39 | 15.32 | 16.85 | 9 | 24.32 | 14.65 | 27 | 0.04 |
| 40-44 | 14.96 | 10.42 | 8 | 27.36 | 11.97 | 5 | 0.06 |
| 45 | 17.80 | 11.16 | 16 | 33.69 | 13.68 | 10 | 0.002 |
| On Medication | 13.98 | 12.0 | 54 | — | — | — | — |
| Off Medication | 18.20 | 7.36 | 16 | — | — | — | 0.001 ² |
| First-degree relatives | 21.15 | 12.7 | 23 | — | — | — | 0.1 ² |

¹MAO activity is expressed as nanomoles of benzylamine utilized per two hours per milligram of protein.

²Compared to controls.

No significant correlation between age and lymphocyte monoamine oxidase for either patients or controls was found, however, white male patients had a trend toward increased lymphocyte monoamine oxidase with age. Since the mean age of the schizophrenic group was 38.3 ± 16.4 and that of the controls was 28.2 ± 11.4 , schizophrenic and control population were also compared by decade. As can be seen in Table 1, patients always had lower lymphocyte monoamine oxidase activity than controls, but the degree of significance of the difference varied, reaching the greatest significance among the 18- to 29-year-olds.

Analysis by racial group (Table 2) shows the mean lymphocyte monoamine oxidase activity of black males to be significantly lower than that of white males among normal subjects, although not among the schizophrenic group. The monoamine oxidase activity of the black patients is not statistically different from that of black normals.

Table 2

MEAN LYMPHOCYTE MAO ACTIVITY BY RACE AND SEX¹

| | Patients | | | Controls | | | p value |
|---------------|----------|-------|----|----------|-------|----|---------|
| | Mean | SD | n | Mean | SD | n | |
| Blacks | 14.86 | 12.0 | 23 | 20.84 | 14.88 | 30 | NS |
| Whites | 14.99 | 11.46 | 37 | 28.11 | 15.78 | 84 | 0.001 |
| Black males | 14.95 | 10.60 | 14 | 15.89 | 8.59 | 22 | NS |
| White males | 14.65 | 10.84 | 30 | 30.04 | 16.84 | 66 | 0.001 |
| Black females | 14.7 | 14.5 | 9 | 34.47 | 20.16 | 8 | 0.03 |
| White females | 18.5 | 14.0 | 10 | 24.69 | 12.0 | 17 | NS |

¹MAO activity is expressed as nanomoles of benzylamine utilized per two hours per milligram of protein.

The mean lymphocyte monoamine oxidase determined for all patients drug-free for at least three weeks was not statistically different from that for those on stable doses of neuroleptics. In 10 patients who had serial lymphocyte monoamine oxidase determinations both on and off medication, four patients' activities decreased on medication, three increased, and three stayed the same.

When the 62 chronic schizophrenic patients were divided into those with paranoid features and those without, the chronic undifferentiated schizophrenic patients with paranoid features did not significantly lower lymphocyte monoamine oxidase activity than that of patients with chronic undifferentiated schizophrenia without paranoid features.

These results confirm the finding of low monoamine oxidase activity in lymphocytes of chronic schizophrenic patients. The finding of decreased monoamine oxidase activity in a tissue other than platelets adds importance to the association between low monoamine oxidase and schizophrenia.

The racial difference in lymphocyte monoamine oxidase activity found in our normal population is statistically significant. Although questioned before in the platelet monoamine oxidase literature, this issue has never been fully investigated. Our white population shows a clear difference between patients and controls, but our black population does not. It is known that alcoholism, anemia, migraine headaches, estrogen therapy, thyroid hormone or testosterone abnormalities, and family history of psychiatric illness are also associated with low monoamine oxidase activity. These factors were considered when we prescreened our normal population, so they would not appear to be responsible for the racial difference observed.

Women have been reported to have higher mean platelet monoamine oxidase activity than males. In addition, monoamine oxidase activity in females shows a variation related to the menstrual cycles of about 23%. We found a greater variation in activity among the female patients and controls than among the males, which may also have led to the lower statistical p values in our relatively small group of females.

2. Binding in B-Cell Lymphocytes

Because specific ^3H -spiroperidol binding has been demonstrated on B-cell lymphocytes, the question arises of whether this binding may be altered in such diseases as Parkinsonism or schizophrenia. To begin addressing this question we are presently developing an assay that would be appropriate to schizophrenic patients that would be a convenient peripheral marker in CSF.

Once this assay is perfected, we plan to use it to follow schizophrenic patients, both on and off medications, to investigate the effects of neuroleptics on bindings and to see if binding is a state phenomenon that varies over time.

B. Animal: Peripheral Measures

1. Conjugated Catecholamines in Rat Spinal Cord

There are a number of reports that indirectly suggest a role for catecholamine conjugation in the overall mechanism of central inactivation of catecholamines. Of the two known major conjugation mechanisms that occur in mammals; glucuronidation and sulfation, the latter is believed to exist only in the central nervous system. The enzyme responsible is

phenolsulfotransferase which is widely distributed in both the brain and the spinal cord. Because of its non-specific nature, this enzyme also conjugates drugs and a host of other endogenous substances beside the catecholamines. Furthermore, its *in vitro* ability to readily sulfate conjugate biogenic amines suggest a similar *in vivo* property. In spite of this background of knowledge, information on the occurrence of conjugated catecholamines in the central nervous system is conspicuously lacking. In an attempt to bridge this gap in our knowledge, we have utilized a recently developed mass fragmentographic method to measure both total and conjugated catecholamines in the brain and spinal cord.

The percentages of the total catecholamines that were conjugated in the spinal cord are appreciably higher than that in the hypothalamus. However, the percentages of conjugated dopamine and norepinephrine in the five different spinal regions analyzed were comparable. In both the cervical and lumbar regions of the cord, the ventral horns showed higher percentages (not statistically significant) than the conjugated amines.

The effects of three classes of drugs; amphetamine, desmethylinipramine and haloperidol on hypothalamic and cervical spinal cord catecholamines are shown in Table 1. None of these drugs significantly changed the concentration of the conjugated or total catecholamines in the spinal cord. Of the three drugs tested, only amphetamine significantly elevated hypothalamic conjugated NE.

Table 1

THE EFFECTS OF DRUGS ON HYPOTHALAMIC AND CERVICAL CORD TOTAL
AND CONJUGATED CATECHOLAMINE

Results are expressed as mean \pm SEM for five separate rats

| | | NOREPINEPHRINE (ng/mg protein) | DOPAMINE (ng/mg protein) | |
|--|--------------|-----------------------------------|-----------------------------|-------------------|
| <u>TREATMENT</u> | <u>TOTAL</u> | <u>CONJUGATED</u> | <u>TOTAL</u> | <u>CONJUGATED</u> |
| <u>Control</u> | | | | |
| Hypothalamus | 24.6 + 0.9 | 3.49 + 0.13 | 5.57 + 0.20 | 1.09 + 0.26 |
| Cervical Cord | 5.98 + 0.41 | 1.08 + 0.11 | 0.90 + 0.05 | 0.20 + 0.04 |
| <u>Amphetamine^a</u> | | | | |
| Hypothalamus | 22.7 + 0.5 | 4.39 + 0.39* | 6.27 + 0.69 | 1.23 + 0.19 |
| Cervical Cord | 5.68 + 0.98 | 1.32 + 0.17 | 0.97 + 0.09 | 0.21 + 0.18 |
| <u>Desmethylinipramine^b</u> | | | | |
| Hypothalamus | 27.6 + 1.5 | 3.15 + 0.06 | 7.33 + 1.45 | 0.97 + 0.07 |
| Cervical Cord | 7.0 + 0.51 | 1.07 + 0.03 | 0.93 + 0.11 | 0.18 + 0.04 |
| <u>Haloperidol^c</u> | | | | |
| Hypothalamus | 27.5 + 2.0 | 3.75 + 0.03 | 6.83 + 0.66 | 1.35 + 0.25 |

| | | | | |
|---------------|-----------------|-----------------|-----------------|-----------------|
| Cervical Cord | 6.14 \pm 0.49 | 1.19 \pm 0.16 | 0.91 \pm 0.01 | 0.17 \pm 0.02 |
|---------------|-----------------|-----------------|-----------------|-----------------|

*p < 0.05

a5 mg/kg, rats killed after 45 minutes

b10 mg/kg, rats killed after 60 minutes

c1 mg/kg, rats killed after 90 minutes

The results offer the first evidence of a substantial *in vivo* conjugation of catecholamines in the rat hypothalamus and spinal cord. The extent of amine conjugation in these two organs, however, are not similar. For example the percentages of conjugated NE and DA in all five spinal regions studied were higher than those in hypothalamus. Other differences include a relatively higher percentage of conjugated DA than NE in the hypothalamus but apparently not in the spinal cord.

2. Phenylethylamine (PEA), 5HT, 5HIAA and Tryptophan

Phenylethylamine (PEA), an endogenous non-catecholic monoamine, can produce similar behavioral effects in the rat as amphetamine (AMPH). These effects may be due to overactivity of brain serotonergic system. Since the effects of PEA on central serotonin (5HT) metabolism are poorly known, we examined the influences of acute and chronic PEA treatments on the concentrations of 5HT, 5-hydroxyindoleacetic acid (5HIAA) and tryptophan in rat hypothalamus and caudate nucleus, and compared them with the influences of AMPH treatments.

Sprague-Dawley rats weighing about 200 g were used. PEA (100 mg/kg) and d-amphetamine sulfate (5 mg/kg) were administered intragastrically (i.g.). The brains were quickly removed, rinsed in ice-cold saline, the hypothalamus and caudate nuclei were dissected. The brain parts were immediately frozen on dry ice, and stored at 210° K until analyzed. To assess the chronic effects of PEA and AMPH, the animals were treated twice daily for 10 days with PEA (100 mg/kg, i.g.) and AMPH (5 mg/kg, i.g.). The brain parts were dissected out two hours after the first injection on Day 10. Furthermore, five other groups of rats were given acute intravenous (i.v.) injections with saline or PEA (5 to 40 mg/kg) and the brain parts removed 20 minutes later for biochemical measurements.

Hypothalamic and caudate 5HT and 5HIAA concentrations were determined using reversed phase liquid chromatography with electrochemical detection. Brain samples were weighed, homogenized with a Tekmar STT polytron (at maximal setting for 5 s) in about 14 volumes of ice-cold 0.2 M percholic acid, and centrifuged for eight minutes at 16,000 x g (Sorvall RC-5B) at 275° K. Six fluoroserotonin was added into the samples before the homogenization step to serve as an internal standard. One hundred microliter of the supernatant was injected into the high pressure liquid chromatograph (HPLC). The concentrations were calculated from the peak height ratios of the standards and samples to the internal standard. Tryptophan content was measured from the same extracts of the brain. The retention factor for tryptophan in this system was 4.3, and the fluorometer response was linear over the range of 5 to 25 pmol. Concentration of tryptophan in the tissue samples was calculated from the height of the sample peak in reference to the peak height line prepared with standards.

Acute i.g. administration of PEA and AMPH produced different changes in hypothalamic and caudate concentrations of indolic substances. PEA increased the concentration of 5HIAA, but had no effects on 5HT or tryptophan concentrations. AMPH increased only the tryptophan concentration. These animals were examined two hours after drug administra-

tion, when PEA concentrations in the brain has returned to the baseline. We also measured the concentration of 5HT, 5HIAA and tryptophan 20 minutes after i.v. injections of various doses of PEA. In spite of the fact that brain PEA level at this time is markedly elevated, no consistent, dose-dependent changes could be seen in any of the measured substances; a slight increase in tryptophan in the hypothalamus was, however, detected at two doses.

Chronic treatment with PEA and AMPH failed to change brain 5HT and 5HIAA concentration, but AMPH increased the tryptophan concentration of chronically treated rats. Two hours after the last injection, PEA concentration in the brain had returned to the baseline, whereas AMPH concentration remained high.

C. Minerals in Central Cerebrospinal Fluid

1. Copper

Copper is an essential trace element with many effects in humans. It is intimately involved in brain myelination. Copper functions as a cofactor in dopamine-beta-hydroxylase, involved in the conversion of dopamine to norepinephrine. These catecholamine neurotransmitters have been linked to the mechanism of action of antipsychotic drugs, to neuropsychiatric drugs and to neuropsychiatric pathology. A number of authors have reported elevated concentrations of serum copper in some schizophrenics. However, such elevations in serum copper concentrations may be a nonspecific finding, since similar increases are associated with myocardial infarction, stress, lymphoma, leukemia, iron deficiency and other dietary factors, hepatic disease, renal disease and acute and chronic infections.

In our work investigating the role of copper in schizophrenia we studied normal controls who showed no significant medical, psychiatric, and substance abuse disorders. Another group was recruited from subjects with a history of heroin addiction. These subjects had not taken drugs for a period of two to four months and were living in a therapeutic community on the hospital grounds. The schizophrenic patients received the same diet as the ex-heroin addicts. Diagnoses were based on RDC criteria; criteria for paranoid and "paranoid features" subgroups. Unmedicated patients had been off neuroleptics for three to four weeks. Medicated patients had been treated with neuroleptics for at least one month, and many had been on medications for one to 10 or more years. The medicated and unmedicated schizophrenic patients did not differ as to average length of hospitalization. Since oral contraceptives may alter compartmentalization of copper, we determined whether these subjects had been on such drugs when CSF was obtained; none of the schizophrenic patients took oral contraceptives, while only one normal volunteer did.

No significant differences in CSF copper concentrations were found between drug-free schizophrenic patients, schizophrenic patients on neuroleptics, ex-heroin addicts, and normal controls (see Table 1). There were also no significant differences in CSF copper between blacks and whites. Female schizophrenic patients had significantly lower CSF copper concentrations than male schizophrenic patients (see Table 2); however, there were no differences between male and female normal controls. The unmedicated schizophrenic females had the lowest CSF copper concentrations, but the difference between them and the female normal controls (5.9 ± 1.6 versus 7.6 ± 1.5 ppb, $t=1.97$, $p=.07$) was not statistically significant. The difference between unmedicated versus medicated female schizophrenic patients likewise was not significant (5.9 ± 1.6 versus 7.4 ± 1.5 ppb, $t=1.74$, $p=.11$). The one control subject taking an oral contraceptive had a CSF copper of 6 ppb, which is one standard deviation below the mean of 7.6 ppb for all female normal controls.

Table 1

CSF COPPER CONCENTRATION AND DEMOGRAPHIC
CHARACTERISTICS OF SCHIZOPHRENIC PATIENTS AND CONTROLS

| | N | Mean Age | Race (B/W) | Gender (M/F) | CSF Copper in ppb (Mean + Standard Deviation) |
|---|----|----------|---------------|-----------------|---|
| Drug-Free Schizophrenic Patients | 28 | 29.8 | 11/17 | 20/8 | 7.3 \pm 1.8 |
| Schizophrenic Patients Neuroleptics | 23 | 30.2 | 20/3 | 18/5 | 7.9 \pm 1.9 |
| Normal Controls | 14 | 33.0 | 13/1 | 9/5 | 7.5 \pm 3.1 |
| Ex-Heroin Addicts | 10 | 27.7 | 9/1 | 8/2 | 6.9 \pm 1.6 |

Table 2

GENDER DIFFERENCES IN CSF COPPER

| | All Schizophrenic Patients | | Unmedicated Schizophrenic Patients | | Normal Controls | |
|--------------------------|-------------------------------|--------|--|--------|--------------------|--------|
| | Male | Female | Male | Female | Male | Female |
| Mean CSF Copper (ppb) | 8.0 | *6.5 | 7.9 | **5.9 | 7.4 | 7.6 |
| Standard Deviation | 1.7 | 1.7 | 1.6 | 1.6 | 3.71 | 1.5 |
| N | 38 | 13 | 20 | 8 | 9 | 5 |

*t=2.86, df=49, p=0.006

**t=2.98, df=26, p=0.007

We were unable to find any evidence of CSF copper gradient. First and last ml of CSF from three patients were analyzed and no trend for increasing or decreasing copper concentration was found. Correlation coefficients showed no significant relationship between CSF copper and duration of hospitalization or platelet monoamine oxidase activity. Subtyping by diagnosis (paranoid, paranoid features, or chronic undifferentiated schizophrenia) or CT scan abnormalities did not reveal any differences in CSF copper concentrations.

B. Zinc

Although zinc is a trace metal comprising about 2.5 grams of total body mass, it is the fourth most prevalent cation in the brain (following sodium, potassium and magnesium). Zinc plays an essential role in several enzyme systems concerned with protein synthesis, DNA replication and repair, and the stabilization of biological membranes. It directly affects synaptic transmission, and inhibits the uptake of norepinephrine and choline into brain synaptosomes.

Zinc has been implicated in several aspects of brain metabolism. Prenatal zinc deficiency in experimental animals reportedly induced aggressiveness and persistent learning disability. A role has been proposed for zinc in the development of psychiatric symptoms when they found high urinary zinc in a patient with porphyria. It has been hypothesized that increased urinary zinc excretion resulted in zinc deficiency which produced psychiatric symptoms typical of the disease. It has been shown, also, that increased urinary excretion of zinc in patients with porphyria is associated with uroporphyrin binding of zinc. Acute zinc depletion in humans has also been produced by oral administration of the amino acid L-histidine and the emotional lability, ideas of reference, acute depression, and cerebellar dysfunction that accompany the zinc depletion are dramatically reversed within 24 hours of zinc replacement therapy despite continued administration of L-histidine. Zinc deficiency associated with parenteral hyperalimentation can result in paranoia and confusion, which is also rapidly reversed by zinc replacement therapy. In premature infants, zinc deficiency may also cause irritability and inappropriate crying.

We have previously found no significant differences in zinc concentrations in blood, urine, brain and genetic aspirate in unmedicated schizophrenic patients and our own controls. Zinc concentrations in hair, blood, and urine, however, may not reflect brain or total body zinc status. In an attempt to obtain another index of body zinc, more clearly related to cerebral zinc metabolism, we measured cerebrospinal fluid (CSF) zinc concentrations in ex-heroin addicts, schizophrenic patients, and normal subjects.

We studied three groups of subjects: schizophrenic patients, normal controls and ex-heroin addicts (Table 1). There were fifty-one schizophrenic patients, eight had been drug-free for three to four weeks prior to the study; the other twenty-three were on conventional neuroleptic medication. Fourteen normal volunteers were recruited, most were hospital employees. All were in good health and found to be without significant medical, psychiatric or substance abuse disorders. In addition, 10 patients with a history of opiate abuse were studied. They had been drug-free for two to four months and were living in a therapeutic community located on the same hospital grounds at the time of the study. All hospitalized patients received identical diets. None of the subjects were on dietary zinc supplements.

Table 1
CEREBROSPINAL FLUID ZINC CONCENTRATIONS
(MEAN \pm SD)

| | Drug-Free Schizophrenics Patients | Neuroleptic Treated Schizophrenics Patients | Ex-Heroin Addicts Patients | Normal Controls |
|-----|---|--|----------------------------------|--------------------|
| (N) | 28 | 23 | 10 | 14 |

| | | | | |
|-----------|--------|--------|-------|--------|
| Age+ | 29.8 | 30.2 | 27.7 | 33.0 |
| Race W | 17 | 3 | 1 | 1 |
| B | 11 | 20 | 9 | 13 |
| Sex M | 20 | 18 | 8 | 9 |
| F | 8 | 5 | 2 | 5 |
| CSF Zinc* | 21.1 | 32.2 | 19.7 | 31.5 |
| SD | (10.0) | (16.5) | (4.2) | (19.0) |

+ANOVA, NS

*ANOVA, $df(3,34)=4.83$, $p<.007$, Welch Approximation

Zinc was determined by limited flame aspiration atomic absorption spectrophotometry on an Instrumentation Laboratory (Lexington, MA) 951 atomic absorption spectrophotometer. A deuterium continuum light source covering the range from 200-330 nm was used in all analyses. The zinc lamp was set at a wavelength of 213.9 nm. For each measurement, 100 μ l CSF was aspirated from a small teflon cup attached to the stainless steel capillary tube of the nebulizer of the instrument and the signal (peak height) of the sample recorded. Atomization was carried out using a single slot Belling burner with an air-acetylene flame. Signals were displayed on both a cathode ray tube screen and on a microcomputer controlled paper tape operated by a high speed printer. Standard curves for 10-100 ppb zinc were linear. Duplicate sample variation was \pm 3%, standard additions of 10-100 ppb zinc did not vary more than 5%.

Analysis of variance (ANOVA) was used to ascertain overall differences between groups. When the ANOVA demonstrated an overall significant difference between groups, individual comparisons between groups were made using a two-tailed Student's t-test when the variances were equal or a two-tailed Fischer-Beheh's t-test when comparisons were made between groups with unequal variances.

Fischer's Exact Probabilities were calculated to ascertain the significance of the distribution of group values about the median CSF zinc concentration. Analysis of covariance was used to evaluate the contribution of race and group membership to the overall difference. Pearson product moment correlations were also determined when appropriate.

The overall ANOVA of the four groups studied (medicated and unmedicated schizophrenic patients, ex-addicts, and normal controls), was significant ($p=.007$, $F=4.83$, $df=3,34$, Welch approximation for unequal variance, see Table 1).

CSF zinc concentration was significantly lower in the ex-addict patients (mean=19.7 μ g/L) than either the normal controls (31.5 μ g/L, $p=.05$, $t=2.17$, Fischer-Beheh's t-test) or the neuroleptic treated schizophrenic group (32.2 μ g/L, $p=.002$, $t=3.39$). The ex-addicts did not differ significantly from the drug-free schizophrenics ($t=1.18$).

While CSF zinc concentrations were significantly lower in the drug-free schizophrenics (21.1 μ g/L) than the neuroleptic treated schizophrenics (32.2 μ g/L, $p=.005$, $t=2.80$), the drug-free schizophrenic patients did not differ significantly from the normal controls ($p=.085$,

$t=1.83$). It should be noted that CSF zinc concentrations were significantly greater in black than in white subjects (29.3 ug/L versus 19.1 ug/L, $t=4.05$, $p < .001$), and most of our subjects were black, especially among the medicated schizophrenic, normal control, and ex-heroin addict groups. The majority of drug-free schizophrenics, however, were white, and within that group, black patients also tended to have higher CSF zinc concentrations than white patients (25.5 ug/L versus 18.4 ug/L, $t=1.93$, $p=.065$).

When data from black subjects only were analyzed, the overall ANOVA was significant ($p=.006$, $F=5.48$, $df=3,24$, Welch). Of the black subjects, the ex-heroin addicts had CSF zinc concentrations significantly lower than either the normal controls ($p=.05$, $t=2.12$) or the medicated schizophrenic patients ($p=.001$, $t=3.82$). Black unmedicated schizophrenic patients were not significantly different from either the black normal controls or the black medicated schizophrenic patients.

CSF zinc concentrations were not different in males compared with females, and were not correlated with age or length of hospitalization. In addition, CSF zinc concentrations were not significantly different in schizophrenics with tardive dyskinesia (5 of 20 patients in whom this was ascertained) or in patients with and without ventricular enlargement on computerized tomography (12 and 15 patients, respectively). Paranoid and nonparanoid subtypes did not differ.

D. Post Mortem Studies

1. Catecholamines

Catecholamines have been hypothesized to play an important role in the schizophrenic syndrome. Initial reports of increased dopamine (DA) concentrations in nucleus accumbens of schizophrenic patients have not been confirmed by other studies. Differences in brain dissection and the subtypes of the patients are two factors which may contribute to discrepancies. Regardless, concentrations of metabolites of DA appear to be normal suggesting there is no increase in turnover. Previous reports of an increase in the hypothalamus of 3-methoxy-4-dihydroxyphenylglycol (MHPG), a NE metabolite, did not make comparisons by subtyping because of the small sample size. The following work, therefore, examined concentrations of DA, NE and their metabolites in the nucleus accumbens and hypothalamus of chronic schizophrenic patients subtyped using Research Diagnostic Criteria.

Brains were collected and dissected. Catecholamines and their metabolites were measured with gas-chromatography mass spectrometry. Protein was measured by the Lowry method. Diagnoses were made after chart reviews, or in two cases, after interviews with people familiar with the subject. Subjects were placed in one of four groups which included normal controls, patients with chronic paranoid schizophrenia, patients with chronic undifferentiated schizophrenia and patients with other psychiatric disorders. These groups were reasonably well-matched for age, gender, race and postmortem interval. All of the normals were drug-free at autopsy as were 17 of the patients. Three of the patients never received neuroleptics, but none of these subjects suffered from schizophrenia. Diagnoses and biochemical determinations were made by separate investigators blind to each others' results. Results were analyzed by parametric statistics.

There were no significant differences between patients and controls in the nucleus accumbens or hypothalamus with respect to concentrations of DA, homovanillic acid (HVA) or dihydroxyphenylacetic acid (DOPAC) (see Tables 1 and 2).

Table 1

CATECHOLAMINES AND METABOLITES (MEAN + STANDARD DEVIATION;
NUMBER (N; NG/MG PROTEIN) IN HUMAN NUCLEUS

| | CPS | CUS | Others | Normals |
|--------------------|------------------------|---------------------|--------------------|----------------------|
| DA | 65.1 + 18.4 (10) | 51.3 + 13.4 (8) | 66.7 + 22.3 (8) | 59.8 + 34.5 (13) |
| HVA | 104.5 + 31.9 (10) | 105.7 + 14.7 (6) | 93.4 + 20.6 (7) | 90.5 + 17.5 (13) |
| DOPAC | 6.89 + 2.11 (10) | 9.59 + 3.50 (7) | 9.51 + 3.57 (7) | 10.37 + 6.00 (14) |
| NE | 2.54 + 1.62* (10) | 0.99 + 0.69 (8) | 0.56 + 0.35 (8) | 0.82 + 0.60 (12) |
| Free MHPG | 0.46 + 0.18 (10) | 0.40 + 0.17 (8) | 0.44 + 0.46 (8) | 0.30 + 0.16 (14) |
| Conjugated MHPG | 1.15 + 0.62** (10) | 0.75 + 0.37 (7) | 0.70 + 0.46 (8) | 0.47 + 0.19 (13) |
| Total MHPG | 1.61 + 0.77*** (10) | 1.06 + 0.46 (8) | 1.14 + 0.46 (8) | 0.71 + 0.21 (13) |

*F(3,34)=7.51, $p < 0.001$; $p < 0.01$ relative to CUS, Others and Normals

**F(3,34)=4.96, $p < 0.006$; $p < 0.01$ relative of Normals

***F(3,35)=5.49; $p < 0.004$; $p < 0.01$ relative to Normals

Table 2

CATECHOLAMINES AND METABOLITES (MEAN + SD (N);
NG/MG PROTEIN) IN HUMAN HYPOTHALAMUS

| | CPS | CUS | Others | Normals |
|-------|---------------------|---------------------|---------------------|----------------------|
| DA | 1.84 + 1.85 (10) | 3.30 + 4.14 (10) | 2.68 + 3.22 (9) | 10.81 + 13.0 (17) |
| DOPAC | 1.08 + 0.67 (11) | 0.50 + 0.07 (9) | 0.82 + 0.54 (9) | 1.57 + 1.69 (15) |
| HVA | 31.0 + 10.8 (11) | 35.1 + 8.2 (9) | 30.6 + 12.6 (10) | 36.3 + 21.4 (16) |

| | | | | |
|-----------|------------------------|---------------------|--------------------|---------------------|
| Free MHPG | 4.55 + 2.71*** (11) | 2.75 + 1.90 (10) | 2.95 + 1.75 (9) | 1.13 + 1.04 (16) |
|-----------|------------------------|---------------------|--------------------|---------------------|

*F(3,43)=5.47, $p < 0.004$; $p < 0.01$ relative to Normals

** $p < 0.05$ relative to Normals

***F(3,42)=6.74, $p < 0.002$; $p < 0.01$ relative to Normals

Norepinephrine concentrations in the nucleus accumbens of chronic paranoid schizophrenics were increased relative to all other groups (one-way analysis of variance F(3,34)=7.51, $p < 0.00$; $p < 0.01$, Neuman-Keuls test). In these same patients there were also increase in total MHPG in the nucleus accumbens relative to normal controls (F(3,35)=5.49, $p < 0.004$; $p < 0.01$). Free MHPG in the nucleus accumbens was not increased in the chronic paranoid schizophrenic group (see Table 1). A similar picture was seen in the hypothalamus where NE concentrations were increased in both chronic schizophrenic groups relative to normals (F(3,43)=5.47, $p < 0.004$; $p < 0.01$). Free MHPG in the hypothalamus was increased in chronic paranoid schizophrenics relative to normals only (F(3,42)=6.74, $p < 0.002$; $p < 0.01$) (See Table 2).

A number of other variables such as age, gender, race, and postmortem interval from death to autopsy were examined in relation to DA, NE, DOPAC, HVA and MHPG both in normal controls and all the subjects in the study. Although several significant correlations emerged they did not account for the observed differences. A comparison with patients who were drug-free at autopsy and those who were on neuroleptics at the time of death yielded no significant findings.

These results lend no support to the initial reports of increased DA concentrations in the nucleus accumbens of chronic schizophrenic patients. These results do support the finding of increased NE concentrations in the nucleus accumbens of chronic paranoid schizophrenics. As has been shown elsewhere, norepinephrine concentrations in the nucleus accumbens vary widely according to the dissection. Regardless, chronic paranoid schizophrenic patients appear to have increased MHPG as well. Since the increase in MHPG in the nucleus accumbens is not free, it appears that there is increased conjugated MHPG. Although patients who were drug-free at autopsy were among those with increased NE and MHPG in the nucleus accumbens, this does not rule out a long-term effect of neuroleptics. Nevertheless, chronic undifferentiated schizophrenics and several of the patients in the other psychiatric disorder group received chronic neuroleptic treatment. If this is a drug effect, it does not appear to occur in all of the patients.

2. D2 and D3 Receptor Binding

Research has found an elevation in D2 (post synaptic) binding in schizophrenic brains. Some have suggested that this binding may be a drug effect. The studies examining D3 (auto-receptor) binding have concluded that these are normal in schizophrenics. Neuroleptics, however, can increase D3 binding. Therefore, there is the possibility that the normal findings of D3 bindings in schizophrenic brains may represent a drug effect on initially subnormal D3 binding.

To investigate further, we are examining D2 and D3 receptor binding in human post-mortem brains. Also, we are examining normal D2 binding patients as a way of characterizing those patients who do not have a drug effect and examining these patients further by assessing their D3 binding levels.

III. Pharmacology

A. Clonazepam

Clonazepam is the most potent benzodiazepine receptor and GABA agonist clinically available in the United States. It is an effective anticonvulsant and has been advocated as a treatment for tardive dyskinesia (TD). While testing the efficacy of this medication in chronic schizophrenic patients with TD, we noted a remarkable psychological improvement in a 21-year-old male. Clonazepam treatment was associated with a reduction in assaultive behavior and incoherence of speech, which had continued during 18 months of hospitalization, despite intensive neuroleptic therapy. The patient was discharged on clonazepam (3 mg/24 hours) and haloperidol (40 mg/24 hours) and has lived at home for over one year. This observation, combined with recent reports of the efficacy of diazepam in the treatment of schizophrenia and lorazepam in the treatment of acute psychosis led us to attempt a therapeutic trial of clonazepam in schizophrenic patients.

Thirteen chronic schizophrenic patients (10 males, 3 females; age range 21-45 years) participated in the study. All patients were stabilized on a constant dose of neuroleptics at least two weeks prior to the start of the trial. After an initial two week period on placebo, clonazepam was begun at a dose of 1 mg/24 hours as a single h.s. dose. The dose was increased by 1 mg every 5th day until persistent untoward effects, usually sedation, appeared. The dose was then adjusted downward to the "optimal" dose (the maximum dose tolerated without side effects), and continued for 28 days. After tapering the dose over a 14-day period, patients received matched placebo clonazepam for two weeks. Patients and staff were blind to medication status. Behavioral ratings were performed twice daily by trained nursing staff using a scaled modified version of the Brief Psychiatric Rating Scale (BPRS).

Nine patients completed the entire protocol. There was no significant effect of clonazepam on the symptoms of this group of patients. Four patients had aggressive behavior. Clonazepam was not efficacious as an adjunctive treatment in chronic schizophrenic patients. Since clonazepam is a powerful GABA agonist these findings do not support a patients demonstrated aggressive behavior during treatment with clonazepam. In two cases, the aggressive acts occurred during clonazepam withdrawal. In the other two cases aggression occurred during increases in the dosage of clonazepam. The first two cases may represent aggravation of psychotic behavior by benzodiazepine withdrawal, whereas the latter two cases may represent a different process or be coincidental.

B. Bromocriptine

Studies postulating a dopaminergic (DA) autoreceptor effect of low-dose apomorphine in man have been inferential, based on decreases in psychosis or increases in sedation. We recently demonstrated that a low-dose of apomorphine (.005 mg/kg) significantly reduced serum homovanillic acid (HVA) concentrations in five medicated schizophrenic patients, two of whom also showed clinical improvement. In view of our results with apomorphine, we have continued our investigation of the suggested DA autoreceptor mechanism by using bromocriptine (BR), an ergopeptine with long acting DA agonist properties.

Eleven (7 males, 4 females) chronic schizophrenics, mean (\pm SD) age of 34.5 ± 7.4 years, receiving a mean daily dose of 1350 mg equivalents of chlorpromazine were given an acute oral dosage of 2 mg of BR or placebo on different days. Clinical assessments were performed using the Brief Psychiatric Rating Scale (BPRS) "blindly" 30 minutes before

treatment and every 30 minutes thereafter for a 2 1/2 hour period. In addition, blood samples were evaluated at those time points for plasma HVA, total MHPG and BR concentrations.

Overall, significant improvement was observed following BR on the total BPRS scores ($p < .01$) and in the subset scores of "hostility" ($p < .03$), "thought disturbance" ($p < .03$) and "miscellaneous symptoms" ($p < .02$). Marked improvement was observed between 30 and 90 minutes, while plasma BR concentrations were greatest between 90 and 120 minutes. A preliminary examination indicates decreases in HVA concentrations following BR.

An acute 2 mg dose of BR clinically improved this group of chronic medicated schizophrenics. Our clinical results with the decrease in plasma HVA concentrations following BR administration may suggest preferential stimulation of the postulated DA autoreceptor.

C. Propranolol

Propranolol may be effective in the treatment of schizophrenia, though not all clinical studies have confirmed this observation. Two possible mechanisms for its antipsychotic property involve its interaction with neuroleptics and its ability to diminish the activity of central nervous system norepinephrine neurons in the laboratory rat. This later finding is of interest in the treatment of schizophrenia because two recent studies of postmortem brains have shown that schizophrenic patients had increased concentrations of norepinephrine, and another study reported cerebrospinal fluid norepinephrine to be elevated in schizophrenia.

To test these hypotheses, we gave propranolol to eleven chronic schizophrenic patients in conjunction with neuroleptics. Serum neuroleptic levels, plasma prolactin and 24 hour urinary 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) were measured. The beta adrenergic blocking property of propranolol was assessed by cyclic AMP induction in lymphocytes by isoproterenol and by monitoring patient pulse and blood pressure.

The study was conducted as a double-blind, placebo controlled trial. Treatment order propranolol-placebo or placebo-propranolol, was determined randomly. After a baseline period of at least one month on the same dosage of neuroleptic medication (treatment mode, haloperidol 30 mg daily), propranolol or placebo was given incrementally over five weeks to a maximum of 1920 mg daily in two divided doses, unless the presence of side effects necessitated a lower maximum. Patients were kept at their highest stable dose for four weeks and then tapered off propranolol over a further four week period.

Behavioral status was evaluated twice daily by a trained nursing staff, blind to medication status of each patient using an expanded version of the Brief Psychiatric Rating Scale (BPRS). Behavioral ratings for the final two weeks of maximal propranolol and placebo treatment period were compared to assess drug response.

We found that propranolol uniformly increased the level of serum neuroleptic. Every patient on neuroleptics had higher levels, as measured by radioreceptor assay, when propranolol was given than when neuroleptic was given alone ($p < 0.005$, Wilcoxon Test).

Plasma prolactin increased in dose dependent relationships when propranolol was given in conjunction with neuroleptics in eight of the nine patients. The results reached statistical significance compared to placebo at a maximum propranolol dose ($t=1$, $p < 0.005$).

Isoproterenol stimulated cAMP production by lymphocytes decreased during propranolol treatment. Examination of 24 hour urinary MHPG and VMA failed to reveal any changes associated with propranolol treatment. Of the nine patients who completed the study, one patient noticed an amelioration of his symptoms while taking propranolol; three noticed an improvement on placebo; and five had no preference.

D. Case Study

Gilles de la Tourette Syndrome (GTS), a disorder marked by multiform motor and phonic tics, behavioral and attentional difficulties, characteristically appears in childhood. Five recent reports describe the adult-onset of GTS in patients receiving neuroleptics, but do not provide biochemical data or pharmacologic responses which might shed light on the etiology of the disorder. We report the case of a 44-year-old male schizophrenic patient in whom tics appeared at age 25 following five years exposure to various neuroleptics. The patient has been maintained on neuroleptics (total life-time dose 5740 gm, mean daily dose 650 mg chlorpromazine-equivalent), and these symptoms have persisted. We examined the effects of acute double-blind administration of apomorphine (0.005 mg/kg) and bromocriptine (2 mg) and open administration of clonidine (0.2 mg) on tics (AIMS inventory), behavior (BPRS), attention (Stroop color-word test), and neurochemistry. Bromocriptine resulted in improved Stroop test performance.

IV. Genetic Studies: The Genain Quadruplets

The role heredity plays in the development of schizophrenia has been disputed for several years, although twin studies and the series of Danish adoption studies strongly suggest an inherited component to schizophrenia. The concordance rate for schizophrenia among twin pairs varies from study to study, although monozygotic twins had consistently greater concordance rates than dizygotic twins.

A rare group of monozygotic quadruplets concordant for schizophrenia were studied extensively in the mid 1950's and the series of psychobiological studies they underwent was the subject of a book. This case study clearly illustrated the complex interplay of genetic and environmental factors that resulted in the diagnoses of schizophrenia for all of the quadruplets.

Now, over 20 years later, we organized a follow-up study of the quadruplets, focusing not only on their clinical and social course, but on biologic variables now known to be relevant to schizophrenia. It could be assumed that differences found between these women would be associated with the environmental inducers of schizophrenia or factors unrelated to the illness, while relevant identical features may give clues to the genetic aspects of the illness.

We examined and observed the quadruplets keeping in mind all of the following hypotheses:

- 1) The "Dopamine Hypothesis": that schizophrenia may be a result of a functional excess of dopamine in parts of the brain.
- 2) The "Norepinephrine Hypothesis": that schizophrenia (particularly the paranoid subtype) is related to chronic excess of brain norepinephrine.

3) The "Enzymatic Defect Hypotheses": that schizophrenia may be related to low levels of enzymes important for the metabolism of the major neurotransmitters.

4) The "Endogenous Hallucinogen Hypotheses": (i.e., phenylethylamine or dimethyltryptamine) that the production of these compounds by schizophrenic patients is related to their symptoms.

5) "The Viral Hypothesis": that an earlier CNS viral infection and the later development of schizophrenia are related.

6) The "Autoimmune Hypothesis": that schizophrenia may be produced by antibodies directed against brain or neural structures.

The quadruplets, in this present group of studies had factors determined that would suggest whether any of the above hypotheses are related to their illnesses, and if so, whether they appear to be predominately environmentally or genetically controlled.

A. Psycho-social and Diagnostic Assessments

Historical and social follow-up data were obtained from serial interviews with all family members. The Schedule for Affective Disorders and Schizophrenia (SADS) and the Psychiatric Assessment Interview (PAI) as well as complete physical and neurological examinations were performed by independent psychiatrists. Life time psychiatric diagnoses were made using Research Diagnostic Criteria (RDC). The Columbia Community Care Schedule was used to assess present and prior social functioning beginning with the onset of illness. Selected variables in the social and community environment of each subject were quantified. The illness of each quadruplet had been stabilized by their home psychiatrist on the following medications: Nora, trifluoperazine 20 mg/24 hours; Iris, fluphenazine decanoate 20 mg IM/q 2 weeks and chlorpromazine 100 qHS; Myra, thiothixene 20 mg qd; Hester thioridazine 200 mg and trifluoperazine 5 mg/24 hours. After a two week period, all four women were hospitalized, all medications withdrawn for a six week period, and mental status was monitored twice daily by trained nurse raters using a modified version of the Brief Psychiatric Rating Scale (BPRS). Mean weekly ratings were examined for absolute scores 4-6 weeks after neuroleptic withdrawal and for evidence of clinical response to neuroleptics prior to discharge. Scores for each item ranged from 0 to 6, with 6 representing the maximum pathology. DSM-III diagnoses were made on each patient during the medication free period by the psychiatrist responsible for their inpatient care.

B. Biochemical Assessments

Blood samples were collected and were drawn twice weekly during the entire evaluation period. In addition, all available family members had blood drawn during the initial assessment for multiple analyses.

Zygosity was established by determining red blood cell antigen types at the NIH blood bank and HLA antigens at the Georgetown University Tissue Typing Department. Monoamine oxidase (MAO) and dopamine-B-hydroxylase (DBH) activities were determined weekly. Serum was analyzed for antinuclear antibodies (ANA) (Clinical Immunology Laboratories, Los Angeles, California) and serial plasma and serum samples stored at -70°C for future determinations to be reported in a later publication.

Alpha-adrenergic receptor function was assessed in platelets from all quadruplets when three weeks medication free and from available relatives during the initial assessment period. The specific binding of an α -receptor antagonist (^3H -dihydroergocryptine) to the α -receptor was quantified and the ability of the α -agonists, norepinephrine, to block prostaglandin E_1 -stimulated cyclic AMP production was measured.

Serum neuroleptic concentrations were determined by assay measuring the ability of neuroleptics to displace ^3H -spiroperidol from its receptor on caudate membranes. Neuroleptic concentrations are expressed as ng/ml chlorpromazine equivalents. Twenty-four hour urine samples were collected twice when all subjects were six weeks drug-free and twice when each subject was stabilized on neuroleptic medication. One 24-hour sample was also collected from the mother. Assays for phenylethylamine (PEA), phenylacetic acid (PAA), DOPAC, HVA, VMA and MHPG were analyzed using mass spectrophotometric procedures.

After extensive tests and observations we feel that although it is clear that these four monozygotic women all have severe mental illnesses, they not only vary in clinical manifestation and severity of the illness, but in response to pharmacological treatment, as well as other biochemical measures relevant to schizophrenia.

The original NIMH diagnosis of each quadruplet in 1955 was schizophrenic reaction, catatonic type. On follow-up, however, the diagnoses appear more variable, although this may be a sign of changed diagnostic criteria used by psychiatrists, rather than changes in the patients' actual illness. All of the sisters undoubtedly have a chronic debilitating mental illness. All of them, at one time, were depressed, paranoid and delusional, specifically with somatic concerns. The severity of their illness and how they adjusted to their environmental circumstances varied considerably. It is not, therefore, surprising that their present life time diagnoses are not the same. Although the biochemical measures obtained vary among the quadruplets, they do not appear related to diagnoses or severity of illness in these patients.

In order to test the relevance of "The Dopamine Hypothesis" of schizophrenia to the disorders present among the quadruplets, urinary and cerebrospinal fluid metabolites of dopamine, DOPAC and HVA, were quantified. These did not differ significantly from normal concentrations in urine for any of the quadruplets, although, urinary excretion of DOPAC tended to be low in all four. While control values were not available for our CSF assays, there appeared to be considerable variance in concentrations of the metabolites among the quadruplets—the most paranoid (Iris) had higher norepinephrine and HVA concentrations than the other three.

None of the quadruplets or their relatives had MAO activity significantly lower than age matched controls. Dopamine- β -hydroxylase (DBH) was found to be consistently lower in all quadruplets and family members compared with controls.

While norepinephrine concentrations were not significantly different from controls, platelet α -receptor number were significantly increased and PGE_1 stimulated cAMP production was reduced in the quadruplets. Since two of the three other family members sampled also had increased α -receptor concentrations and one had decreased PGE_1 -stimulated cAMP production, a genetic component to this abnormality, that may be a marker for the illness, may be hypothesized.

Not only the quadruplets but their mother also had elevated urinary PEA excretion, an indication that further studies might be useful to determine if a genetic alteration in a protein affecting PEA production or degradation is altered in schizophrenia.

Although we can not define the genetic aspects of schizophrenia in a small unusual case study such as this, we can hope to gain some clues using our knowledge of previously published abnormalities in schizophrenia. Three interesting biochemical findings have emerged, however, namely that this biologic family with a high incidence of schizophrenia has significantly elevated urinary PEA excretion, lower plasma DBH activity and higher platelet α -adrenergic receptor concentrations. Since DBH catalyzes the conversion of dopamine to norepinephrine, as well as the metabolism of phenylethylamine to phenylethanolamine and increased PEA can be associated with increases in the α -receptor agonist norepinephrine it is possible that these alterations are interrelated and part of an ill-defined metabolically controlled mechanism.

C. Cognition

Because attentional deficits play an important role in schizophrenia, we examined the quadruplets, also, on their performance on two attentional tasks. The first task was a measure of simple reaction time to an auditory imperative stimulus. Reaction time to four isothermoal trials with 3, 7, and 11 second intervals were imbedded in a randomly ordered series of trials ranging from .5-12 seconds. The crossover measure, quite characteristic of process schizophrenics, is defined as the sum of the differences between the first irregular trial and the average of the three succeeding regular trials of the same interval. The second task, the span of apprehension procedure, requires the subjects to identify the relevant target letters in arrays composed of varying numbers of nontarget letters.

Data for two of the quads were not obtainable off drugs because they were unable to complete the testing. One was tested twice off drugs; and using Cromwell's criterion of a minimum of 1 Omsec crossover, she showed crossover at the 7 sec interval during the first testing off drugs but not during a later off-drug session. Another showed 7 sec crossover off drugs, while on drugs later she did not. One of the two quads tested off drugs showed 10 msec crossover on drugs while the other's crossover was the most extreme.

In the span of apprehension procedures, all quads except one showed more errors with increasing number of distractors (3,7 and 9). They all produced more errors at 9 distractors than a comparable group of normals. Additionally, the overall reaction time for both regular and irregular trials was slower than inpatient process schizophrenics and outpatient populations.

V. Psychophysiology

A. Positron Emission Tomography (PET) and Neuroleptics

Studies of cerebral blood flow in man, and animals studies using autoradiographs of C14 deoxyglucose phosphate have indicated that local cerebral glucose utilization may closely parallel local functional activity. The advent of positron emission tomography (PET) has made it possible to construct three-dimensional maps of glucose utilization in humans almost non-invasively. Regional cerebral blood flow studies in man have demonstrated that sensory perception, voluntary motor activity, as well as different forms of cognitive activity, give rise to local alterations of the cerebral functional ability. Preliminary studies with

the PET have confirmed these findings and indicate that the PET opens entirely new realms of neuropsychological and psychiatric investigation.

Our study investigates changes in local glucose metabolism in schizophrenic patients on and off neuroleptics and provides normal control comparisons. We are attempting to provide new information on the pathophysiology of schizophrenia and on drug action. We expect to find quantitative differences between normal controls and schizophrenics.

The experimental procedure consists of the intravenous administration of 18 F-deoxy-glucose (FDG), a radioactive glucose analog sequential blood sampling for 45 minutes and then the scanning procedure.

Blood samples are drawn through an indwelling venous heparin lock. The samples are drawn from the left hand. The ipsilateral arm is warmed with a water bath to provide arterialized blood.

Approximately 5 mCi of radioactive FDG (15 micrograms/kg) is being administered intravenously. Following the intravenous bolus of tracer, all subjects either rest in a darkened room or perform a 45-minute standard psychological task, the continuous performance task, which commonly differentiates normal and schizophrenic subjects. Patients are tested once when off all psychoactive medication for two weeks or longer, and once when on standard neuroleptic treatment. An interval of at least three months between 18-FDG studies is being observed. Statistical comparisons are being made between (1) schizophrenics on and off medication (as a paired comparison) and (2) improving and nonimproving schizophrenics at baseline, when on drug and baseline/drug differences. Drugs are administered so that double-blind nurse and physician ratings are available. At the conclusion of the task, the imaging procedure is performed, requiring about 10 minutes.

At the present time, little is known about the specific areas of the brain which are involved in the disease and its symptoms. The PET promises to be a technique which permits the study of localized functional activity in specific areas of the living human brain. Thus, this technique has great potential for furthering our understanding of mental illness, tardive dyskinesia, and the mechanisms of action of neuroleptic drugs. We have now begun running subjects and will be reporting our findings when we have analyzed the data.

B. Electrical Activity Mapping (BEAM)

It is generally accepted that schizophrenic patients as a group have a greater occurrence of EEG abnormalities than do normal individuals. There is, however, no consensus as to which, if any, abnormalities predominate or are important. Contributing to this problem has been the difficulty in interpreting the massive quantities of data that are generated by multiple electrode recordings from the human scalp, although numerous ingenious approaches have been suggested.

Recently, advances in solid state technology have produced a technique of brain electrical activity mapping (BEAM) that creates color maps of condensed and summarized EEG or evoked potential data and displays them on an ordinary color television. The technique has been useful in investigating neurologic problems ranging from an anatomically well defined lesion, brain tumor, to a functional deficit, dyslexia. Our work introduces this method to schizophrenia research and demonstrates the applicability of the BEAM technique to the search for biological markers in this disorder.

Twenty-four gold cup electrodes (Grass E56H) were applied to the scalp with collodion according to the standard 10-20 system of placement. The brain electrical activity from these electrodes was amplified through a 20 channel polygraph (Grass 78) and then recorded on a 28 channel FM analogue tape recorder (Honeywell 5600E). The data were processed by computer (Digital Equipment Corporation PDP 11-60). Prior to spectral analysis all raw EEG segments containing artifact were eliminated by visual inspection made on each segment. The data were subjected to a spectral analysis from 0 to 32 Hz on the background EEG using the Fast Fourier transform technique. The signals are filtered tightly at 24db per octave using active Butterworth filters (EEG Associates Mark 4 x 24) in order to avoid 60 Hz and muscle activity artifacts. A determination was made for each electrode of the amount of energy in the EEG bands of delta, theta, alpha and beta.

Each subject was presented with light flashes from a photic stimulator (Grass PS-2) positioned 16 cm in front of the nasion. These flashes are randomly presented by a mini-computer (LSI-11) that also generates trial demarcation on the tape recorder. Visual evoked potentials of 512 millisecond (msec) duration are recorded for analysis. The computer excluded trials that contain muscle or blink artifact. A determination is then made for each electrode of the average evoked potential voltage over 128 epochs each lasting four msec.

The data from the spectral analysis and the visual potentials are retrieved for video display. The analysis of the electrical activity is presented within a graphic outline of the head. Topographic maps of the spectral energy in each of the classic EEG bands and of evoked potential voltage at any four msec epoch after stimulus onset may be presented.

The mean voltage at each of the 20 recording sites is determined for the time interval of interest. Then a 128 x 128 matrix is overlaid upon the head thereby creating 16,384 picture elements. Through linear three dimensional interpolation based upon the values at the three nearest electrodes each of the picture elements around the original electrode values are assigned voltage values. A color scale is then fitted to the values with each color representing a voltage range. The computer has the ability to present new video images every 100 msec. This capability permits the display of brain electrical activity in space as topographic maps as well as time in the form of cartooned images.

To overcome the difficulties inherent in the subjective interpretation of electrical activity maps, a previously developed statistical treatment, significance-probability mapping (SPM) was employed. In this case example, a z-statistic SPM was used.

To demonstrate the possibilities of BEAM, we studied one schizophrenic patient and one control. A case example of the spectral plots of a medicated male chronic schizophrenic patient receiving a neuroleptic displayed the power (in microvolts) of delta, theta, alpha, and beta. For comparison a normal male subject with no medications and negative neurologic and psychiatric history was also presented. Both subjects had eyes closed. The normal subject demonstrated the usual symmetries, and power spectrum analysis that would be expected in normal eyes closed EEG. The schizophrenic subject exhibited striking increases in frontal slow wave activity with little difference in alpha and beta. The significance-probability maps visually localized topographically and assigned magnitudes to differences between subject and control for each EEG band. It should be noted that this case example is presented to demonstrate the technique and is not meant to make a specific statement about schizophrenia. The SPM statistically demonstrates that which is subjectively apparent in the BEAM plots. This case was deliberately chosen to demonstrate differences that are subjectively obvious. The SPM is particularly useful when subjective evaluations are less obvious.

We do suspect, though, that the BEAM technique provides the basis for the determination of EEG biological markers in schizophrenia that may then be overlaid on other complementary topographies thereby raising the possibility of linking electrical activity to anatomic, vascular and metabolic measurements. Such studies are now in progress.

VI. Neuroanatomy: Computerized Axial Tomography (CT)

A. Sibships

Our study of schizophrenic patients by means of computer axial tomography (CT) has progressed throughout the year and has expanded considerably. While it is felt that CT abnormalities are only indirect evidence of underlying pathological processes and that they reveal little or nothing about etiology and pathogenesis, in a recent study, we investigated the relationship of cerebral atrophy to the onset of schizophrenia by comparing the pre-morbid adjustment of 21 patients with atrophy to that of 30 similar patients with normal CT scans. The patients with the CT abnormalities had significantly poorer adjustment during childhood (ages 5-12), suggesting that either the atrophic changes themselves or the processes ultimately responsible for them are early development phenomena and not an acute event in a previously well-functioning individual. Further support for the notion that the CT abnormalities precede the onset of schizophrenia in adult patients comes from the finding that the magnitude of the abnormalities does not correlate with the duration of the illness.

Since the etiologies of neuropathologic abnormalities of childhood are frequently congenital, it follows that the CT abnormalities may have genetic determinants. Also, ventricular size itself may be under genetic control. To investigate these possibilities, we have looked at ventricular size in healthy, asymptomatic sibships and have compared the CT scans of schizophrenic patients to those of their nonschizophrenic siblings. We also report here the prevalence of schizophrenia in first-degree relatives of patients with and without evidence of atrophy.

Ten chronic schizophrenic patients (one female, nine males; mean age \pm SD = 26.7 ± 3.9 ; range 21-35) along with 12 of their nonschizophrenic siblings (six females, six males; age 26.1 ± 6.9 ; range 19-41), volunteered for a noncontrast CT head scan. The patients in this sample were chosen solely because they had available siblings. CT scans of 17 asymptomatic individuals (age 27.9 ± 7.2 ; range 20-42) from seven sibships who had been studied for other research purposes at the National Institutes of Health constituted the controls. The same procedure and machine (EMI CT 1010) were used for all scans.

Evaluation of the scans included quantitative measurement of ventricular size and the size of various cortical structures (viz., sylvian fissure, interhemispheric fissure, cortical sulci) and quantitative assessment of the anterior cerebellar vermis. Quantitation of ventricular size by the ventricular/brain ratio (VBR) method has been shown to be highly reliable. All measurements were made without knowledge of familial relationships. Family histories were obtained by interviewing healthy first-degree relatives of the patients and also of an additional 41 chronic schizophrenic patients who had been scanned previously. Of the 51 family histories thus compiled, 22 were of patients who had CT evidence of atrophy.

Three preliminary findings emerge from this study. First, in healthy asymptomatic individuals there may be a genetic component to the size of the lateral ventricles. This finding should probably be no more surprising than the observation that numerous other physical dimensions have genetic determinants. Nevertheless, for obvious reasons, studying the familial aspects of ventricular size was difficult, if not impossible, before the advent of

CT. If the significant correlation observed is valid--and this is uncertain in view of the small sample--it suggests that the sibs are more alike than would be expected by chance.

Second, the schizophrenic patients had significantly larger ventricles than their non-schizophrenic siblings. Also, none of the siblings had cortical or cerebellar atrophy. Since siblings have many genetic and environmental factors in common with the patient and are close in age, they are a unique control group for confirming the existence of CT abnormalities in schizophrenic patients. This finding also suggests that, at least within these families, those abnormalities that are outside the normal range are markers of the clinical illness and not irrelevant familial traits, or genetic markers of vulnerability.

The third finding is that the siblings of the schizophrenic patients, although all within the normal range, had modestly but statistically significantly larger ventricles than did the controls. The implications of this finding are unclear. It is possible that some genetic predisposition to larger ventricles exists in families of schizophrenic patients.

B. Poor Premorbid Adjustment

In work further investigating ventricular enlargement in some schizophrenic patients, we were interested in whether such abnormalities are related to the pathogenesis of the illness or to non-specific factors to which schizophrenic patients are particularly vulnerable. If the latter possibility is the case, it might be expected that patients with more lengthy illnesses would be more likely to manifest the abnormalities. In studies of patients under age 50 this has not been found. The abnormalities also do not appear to be the result of various treatment modalities, including institutionalization, ECT, or neuroleptic drugs.

To the extent that the CT findings may be related to the pathogenesis of a schizophrenic illness, they might be associated with a distinct manner of onset or premorbid history. For example, if patients with these structural brain abnormalities were found to have had a normal premorbid history, it would implicate an acute postpubertal neuropathological episode, perhaps a viral illness. On the other hand, if patients with the abnormalities appeared to be an impaired group from childhood, then a neuropathological process in early development that predisposed to schizophrenia could be hypothesized. We examined these possibilities by evaluating the relationship between premorbid adjustment and CT abnormalities in schizophrenic patients.

Fifty-one chronic schizophrenic patients (12 women, 39 men; mean age, 29; age range, 20-50) comprised the study sample. Each patient had undergone CT scanning, and measurements from their scans were made of the size of the lateral ventricles and of various cortical fissures and sulci. Twenty-one patients had abnormal scans as defined by a ventricular or sulcal size in excess of the range of 62 similarly aged asymptomatic individuals. Nineteen patients (6 women and 13 men) had enlarged ventricles (ventricular-brain ratio more than 10.6), and eight patients also had dilated sulci. Two of the patients with dilated sulci, both of whom were men, had normal ventricles.

A novel instrument that includes selected items from the Phillips and Gittelman-Klein premorbid adjustment scales and the Elgin prognostic scale was used to assess premorbid adjustment. The rationale for combining these various items into a new format was to develop a scale that would measure adjustment during several specified age periods in addition to scoring overall premorbid adjustment. The new scale has 28 items divided among five subscales, four of which focus on various aspects of social and school functioning during

the following age periods: childhood (younger than age 12), early adolescence (ages 12-15), late adolescence (ages 16-18), and adulthood. In addition, it has a general item scale that attempts to assess the highest level of premorbid adjustment achieved.

One of us who was unaware of the CT scan findings completed the scale for each patient by reviewing the patient's chart. The research charts generally included detailed premorbid histories that were derived from family meetings and records from referral sources. If the charts did not contain sufficient information to complete a particular item, the item was left unrated. To account for unratable items, the score for each age period was expressed as a fraction of the total possible score for the ratable item. Thus the lower scores correspond to better adjustment. Every age period was not ratable in every patient due to insufficient historical data. To help standardize the ratings, the instrument was used by the same individual in an interview format to assess premorbid adjustment of 78 enlisted personnel stationed at an Air Force base in Washington, D.C. Statistical comparisons were done by a two-tailed t-test.

We found that, to the extent that chart reviews and personal interviews are comparable, the premorbid adjustment of the group of 51 chronic schizophrenic patients was significantly worse than that of the Air Force personnel as measured on the subscales during childhood (mean scores \pm SD = $.33 \pm .2$ versus $.23 \pm .1$, $p < .001$), early adolescence ($.44 \pm .2$ versus $.1$, $p < .0001$), late adolescence ($.55 \pm .3$ versus $.17 \pm .1$, $p < .0001$), and adulthood ($.58 \pm .3$ versus $.12 \pm .1$, $p < .0001$), as well as on the general item subscale ($.44 \pm .2$ versus $.09 \pm .1$, $p < .0001$).

The patients with enlarged ventricles scored consistently worse than the other patients; the scores during childhood and on the general item subscale reached statistical significance ($p < .05$). When all the patients with CT abnormalities (the 19 patients with enlarged ventricles plus the two patients with only sulcal dilatation) were compared with the schizophrenic patients with normal scans the differences in premorbid adjustment during early adolescence reached statistical significance as well. Of the nine patients with the poorest adjustment during childhood (those whose rating was more than .50) all had abnormal CT scans. In contrast, of the 13 patients with good childhood adjustment (rating less than .20), only three had abnormal scans ($p < .001$, Fischer exact test).

It is not uncommon in medicine for nonspecific findings to lack clinical significance in one clinical context while being meaningful in another. For example, cortical sulcal dilatation in asymptomatic elderly individuals may be clinically irrelevant while in young patients with systemic lupus erythematosus it is associated with psychosis. Ventricular enlargement and dilated cortical sulci in schizophrenic patients are certainly nonspecific findings, since they are common in the elderly and in many neurological disorders. They have also been described in patients with chronic severe migraine headaches, in alcoholics, in patients with affective disorders and in autistic children. However, since ventricular enlargement is not itself a pathological process but the result of one, it is this underlying process which may be specific.

The results of this study indicate that CT abnormalities in chronic schizophrenic patients are associated with poor premorbid adjustment. Even in a sample of generally poor premorbid patients, the CT scan criteria segregated out a significantly more impaired subgroup. This finding provides further support for the idea that these CT scan findings help define a more homogeneous subgroup of chronic schizophrenic patients.

C. Asymmetries

Various higher cortical functions in human beings, such as handedness, language, musical ability, and spatial perception, appear to be subserved by dominance of one cerebral hemisphere. While these functional asymmetries have been the subject of innumerable studies, the mechanisms underlying them are unknown. The possibility that neuroanatomical asymmetries might, at least in part, be responsible has long been considered, and structural differences between the hemispheres have been noted by many neuroanatomists. Most neuroanatomical studies prior to 1968, however, described relatively minor differences or failed to document quantitatively the magnitude or frequency of the asymmetries. Furthermore, in these early reports, it was difficult to correlate the findings with either normal or pathological brain function. Our studies into the brain's asymmetries, with the aide of computer axial tomography (CT), were designed in an effort to link asymmetrical structure and function in the brain. Our investigations lead us to measure the volume of a major portion of the frontal and occipital lobes of 40 serially sectioned whole brains in the Yakovlev Collection.

The Yakovlev Collection consists of approximately 800 serially sectioned whole brains prepared to exacting and uniform standard. Twenty of the 40 cerebra studied were of fetuses and infants designated as "normative," indicating no evidence of cerebral abnormality. Fifteen cerebra were of fetuses ranging in age from 20 to 42 weeks of gestation. The five infant brains were from babies 3.5 to 8 months old. Within this age range, 32 "normative" cerebra sectioned in the coronal plane were available. Twelve were excluded because either the cerebrum was not intact or the plane of section was clearly not at 90 degrees to the anteroposterior axis. The other 20 cerebra, 14 of which also were designated as "normative," were of four children and 16 adults. The children ranged in age from three to 11 years, the adults from 19 to 98. Of the coronally sectioned normative brains available in this age group, only one had to be excluded because the cerebrum was not intact. Six abnormal adult brains were included that showed evidence of diffuse metabolic brain diseases not known to produce asymmetrical structural changes. These included two cases of hepatic coma, two of Wernicke's encephalopathy, and two of anoxic encephalopathy. Only specimens sectioned in the coronal plane were used, since volume can be more easily determined in brains sectioned in this manner.

To ensure a standardized quantitative method, we selected regions demarcated by unmistakable landmarks. These regions are slightly more generous than those measured in the CT studies. The posterior border of the frontal lobe region was defined by the first section anterior margin of the genu of the corpus callosum. Using a planimeter, an accurate mechanical tool for measuring the area of an irregular space, we determined the cross-sectional area of the left and right hemispheres in this section by tracing along the contours of the cortical gyri. For the adult and child brains, we also measured the area of white matter in each hemisphere by tracing along the internal margin of the cortex. The area of gray matter for the section was derived by subtracting the white matter area from the area of the total hemisphere. Each measurement was made twice, and the mean was used. Initial and repeat measurements were invariably within 3% of the mean. In similar fashion, we measured consecutive sections at 2,800 μ intervals (600 μ intervals for fetal brains) until we reached the frontal pole of the brain. The volume of the region was derived by multiplying the mean of all the areas measured for the region by its length. An analogous method was used to determine the volume of the occipital lobe region although the anterior border of this region was defined by the section closest to the midpoint between the posterior margin of the splenium of the corpus callosum and the occipital pole.

We found that in 32 cases, the volume of the right frontal lobe region was greater than that of the left ($p < 0.01$, binomial test), by an average of 13%. As expected, the volumes

varied considerably from one brain to another, reflecting differences in age and degree of shrinkage (range for adults: left, 11.3 to 48.2 cm, right, 12.4 to 48.0; range for infants and fetuses: left, 0.6 to 12.3 cm, right, 0.7 to 19.7). The volume of the left occipital region was greater than that of the right in 32 of the 40 cases also, by an average of 20%. The volume of the occipital regions also showed considerable variation from one brain to another (range for adults: left, 11.2 to 38.8 cm, right, 9.9 to 31.3; infants and fetuses: left, 0.8 to 15.0 cm, right, 0.8 to 11.2). For the total sample, a paired t-test revealed that the volume of the right frontal lobe was significantly greater than that of the left ($p=0.01$) and the left occipital lobe had a significantly greater volume than the right ($p<0.001$). Twenty-eight cases had larger right frontal and left occipital lobes.

We sought to determine whether these asymmetries had developed subsequent to the acquisition of a lateralized cerebral function such as language or whether they apparently arose during embryological development of the brain. If the former hypothesis were the case, the asymmetries should have been less apparent in the fetal and infant cerebra. This was not found. The frontal volume was greater on the right in 17 of the 20 brains of fetuses and infants, and the occipital volume was greater on the left in 15. A paired t-test for these 20 cerebra showed that the asymmetries were quantitatively significant for both the frontal ($p<0.03$) and occipital ($p<0.02$) regions. A significant effect of age could not be demonstrated. For the 20 cerebra of adults and children, the right frontal and left occipital volumes also were greater in 17 and 15 cases, respectively. This difference in volume approached significance for the frontal asymmetry ($p=0.1$, paired t-test) and was significant for the occipital asymmetry ($p=0.01$) in this small sample of 20 older cerebra.

We also investigated lateralized asymmetries of gray and white matter. Although the differences tended to be in the same direction as for the whole lobes, the only difference that reached significance by paired t-test was the volume of the left occipital gray matter, which was larger than that of the right ($p=0.01$). The ratio of gray to white matter was not significantly different across hemispheres for either the frontal or occipital regions, and the hemispheric gray and white matter volumes were highly correlated (Pearson $r = 0.77$, $p<0.001$) for all regions except the left frontal ($r=0.38$, $p=0.10$).

In addition to the Yakovlev Collection work, we performed a series of studies attempting to better understand these asymmetries and their possible significance in schizophrenia. The subjects were 79 inpatients who met Research Diagnostic Criteria for schizophrenia or schizoaffective psychosis (5 had the latter diagnosis). All had undergone CT brain scanning (EMI Model 1010, 160 x 160 matrix with 8 mm slices). Scans that showed rotational artifact (defined by gross asymmetry of the petrous pyramids) were excluded. The CT scans, in the form of self-developing prints and transparencies, were evaluated to determine occipital asymmetries. The evaluation method involved the use of a cross hair with millimeter calibrations laid on the appropriate CT image. Occipital asymmetry was determined by studying the cut that best visualized the occipital horns of the lateral ventricles and one cut above and one below this level. If the occipital horns were not visualized, we selected a cut through the bodies of the lateral ventricles. The vertical hair was aligned with the inter-hemispheric fissure and the lateral extent of the occipital lobes was measured on the horizontal hair placed 8 mm from the pole. A consistent difference in width of at least 1 mm between the right and left hemispheres on two out of three images was considered as evidence of asymmetry.

We chose to study only the occipital asymmetry and to use a technique that measured asymmetry in three contiguous slices. This was because we found that 1) the determination of frontal but not occipital asymmetries is affected by the degree of brain pathology and 2)

that a measure that uses three contiguous slices gives a higher test-retest reliability. Because this method has not been previously used, we next determined occipital asymmetry in 100 medical and neurological patients whose scans were randomly selected from the radiological files of the NIH. Scans with focal lesions or abnormalities that might produce displacement were excluded. The schizophrenic and medical and neurological scans were read on separate occasions and not blindly. To assess reliability the same rater blindly determined asymmetries on two different occasions on the same 40 control patients. Agreement between the two measures was found to be significant ($p=.019$).

Because reversed asymmetries may be more frequent in left than right-handers and because of the possibility that left-handers may be more frequent in schizophrenia, handedness was assessed in the schizophrenic patients with a 12-item battery. Sixty-six (84%) schizophrenics used their right eye, hand or foot on nine or more items and were considered right-handed; the other 13 (16%) were considered non-right-handed (for simplicity we will call them left-handed). Handedness data were not available on the controls. Statistics were done by a modification of the Fisher Exact Method unless otherwise stated.

There was no difference in the distribution of asymmetries in the 66 right-handed and the 13 left-handed schizophrenic patients (Table 1). Recent investigations in normals have failed to find a difference in the distribution of asymmetries between right and left-handers. We therefore combined right and left-handed schizophrenics and used all 79 patients in our subsequent analyses.

Table 1

OCCIPITAL ASYMMETRIES IN RIGHT-HANDED
COMPARED TO LEFT-HANDED SCHIZOPHRENICS

| | L R "Normal" | L=R | R L "Reversal" |
|---|-----------------|---------|-------------------|
| Left-Handed Schizophrenics (n=13) | 5(38%) | 7(54%) | 1(8%) |
| Right-Handed Schizophrenics | 34(52%) | 20(30%) | 12(18%) |

Fisher Exact Probability = 0.31

There was a significant difference in the distribution of occipital asymmetries ($p=0.018$) between the 79 schizophrenics and the 100 controls (Table 2). Among the schizophrenics compared with the controls, the distribution was shifted to favor an increased frequency of occipital reversals (16% versus 5%).

Table 2

OCCIPITAL ASYMMETRIES IN 79 SCHIZOPHRENICS COMPARED TO 100 CONTROLS

| | L R "Normal" | L=R | R L "Reversal" |
|-----------------------|-----------------|---------|-------------------|
| Neurological Patients | 46(46%) | 49(49%) | 5(5%) |
| Schizophrenics | 39(49%) | 27(43%) | 13(16%) |

Fischer Exact Probability = 0.018

We have shown in previous work that the increased frequency of reversed asymmetry is concentrated in those schizophrenics without CT evidence suggestive of brain atrophy. Because of this finding and since schizophrenics with atrophy might represent a distinct subgroup we divided our patients dependent on whether or not they had evidence of atrophy.

There was a significant difference in the distribution of occipital asymmetries ($p=0.03$) between the 34 schizophrenics with evidence suggestive of atrophy and the 45 schizophrenics with no finding suggestive of atrophy (Table 3). Among those without atrophy, compared to those with atrophy, the distribution was shifted to favor reversed asymmetry (22% versus 9%) at the expense of normal asymmetries (38% versus 68%). When the distribution of asymmetries in these two groups of schizophrenics was compared to that of the controls, it was found to be significantly different for those with ($p=0.02$) and without ($p=0.012$) evidence of atrophy. However, there is reason to believe that an increase in reversals was the important deviation in those without atrophy and an increase in normal asymmetry was important in those with atrophy. If we examine just the frequency the reversals and collapse together the other two categories (normal and no asymmetry) there was a significant difference between schizophrenics without atrophy and controls ($p=0.003$) but no difference between schizophrenics with atrophy and controls ($p=0.4$). On the other hand, if we concentrate on the frequency of normal asymmetry and collapse together the other two categories (reversals and no asymmetry) there was a significant difference between schizophrenics with atrophy and controls ($p=0.046$) but no difference between schizophrenics without atrophy and controls ($p=0.38$).

Table 3

OCCIPITAL ASYMMETRIES IN 45 SCHIZOPHRENICS WITHOUT COMPARED TO 34 SCHIZOPHRENICS WITH CT EVIDENCE OF ATROPHY

| | L R "Normal" | L=R | R L "Reversal" |
|-----------------------------------|-----------------|---------|-------------------|
| Schizophrenics Without Atrophy | 17(38%) | 18(40%) | 10(22%) |
| Schizophrenics With Atrophy | 23(68%) | 8(24%) | 3(9%) |

Fischer Exact Probability = 0.03

Since reversed asymmetries have been associated with lower verbal than performance IQ in the learning disabled and those with developmental dyslexia, we studied this in our schizophrenics. WAIS scores were available on only 23 of the 79 patients. The mean (\pm SD) difference between verbal minus performance IQ for the 14 schizophrenics with normal asymmetry was 11.3 (\pm 9.5), for the five schizophrenics with no asymmetry it was 6.2 (\pm 8.5), and for the four schizophrenics with reversals it was -8 (\pm 12.1). A one-way ANOVA revealed a significant group effect ($F(2,20)=6.11$, $p < 0.01$) and a Scheffe post hoc analysis revealed a significant difference ($p < 0.01$) between the groups with normal and reversal asymmetry.

In normal population cerebral asymmetries are believed to be familial in origin and a post-mortem study found them to be present in fetal brains. To examine whether the reversals in our patients might be on a different basis, possibly secondary to brain insult, we studied the relationship of cerebral asymmetry and lateral ventricular asymmetry. It has been shown that in right-handers the left ventricle is usually larger in 3/4 of the cases. We hypothesized that if occipital reversals in our patients were due to left hemispheric damage, then the left ventricles would be even more frequently enlarged in those with reversed occipital asymmetry than in those without. We determined asymmetry of the lateral ventricles in the following manner: the area of the two lateral ventricles was measured by planimetry on two contiguous CT cuts through the bodies of the lateral ventricles. If there was a greater than .1 cm² difference in area on both cuts it was assumed to be asymmetrical.

Examination of Table 4 shows that larger left ventricles are not more frequent in patients with reversed occipital asymmetry (31%) compared with normal asymmetry (54%). This implies that reversed occipital asymmetries are probably not due to left hemispheric insult.

Table 4

RELATIONSHIP OF OCCIPITAL ASYMMETRY TO LATERAL
VENTRICLE ASYMMETRY IN ALL SCHIZOPHRENICS

| <u>Occipital Asymmetry</u> | <u>Ventricular Asymmetry</u> | | | |
|----------------------------|------------------------------|---------|--------|--|
| | L R | L=R | R L | |
| L R (n=39) | 21(54%) | 15(30%) | 3(8%) | |
| L=R (n=27) | 10(37%) | 15(65%) | 2(7%) | |
| R L | 4(31%) | 5(38%) | 4(31%) | |

Chi Square $p=0.39$

D. CSF 5-HIAA

The hypothesis that serotonin may be involved in the pathogenesis of at least some forms of schizophrenia is one of the oldest neurochemical theories of this disorder. It is based primarily on the fact that the psychedelic drug, LSD, interferes with serotonergic

neuronal systems and on the possibility that aberrant methylation of serotonin could produce endogenous psychotogens such as dimethyltryptamine (DMT) and bufotenin.

Since it is now widely assumed that schizophrenia is not a single disease entity but a biologically heterogeneous collection of possibly distinct subtypes, failure to separate schizophrenic persons into subtypes may explain some of the discrepancy in the CSF 5-HIAA concentrations studies. In our experience, one biological and clinically meaningful subgroup of schizophrenic patients is defined as having subtle morphological brain abnormalities on computed tomography (CT). Those schizophrenic patients whose lateral cerebral ventricles are enlarged on CT scan (at least greater than two standard deviations above mean control values) tend to have poorer performance on neuropsychological testing, more disordered smooth-pursuit eye tracking, poorer premorbid adjustment, and more negative symptoms. This group also may have a poorer response to neuroleptic medication. The relatively poor neuroleptic response raises the question as to whether biogenic amine abnormalities hypothesized in schizophrenia are relevant in this subgroup of patients. To further explore these issues, we report CSF 5-HIAA findings in schizophrenic persons with and without cerebral ventricular enlargement on CT scan.

All inpatient volunteers who underwent CT scans, were less than 50 years of age, and consented to a lumbar puncture were included in the study. All patients met Research Diagnostic Criteria (RDC) for schizophrenia and had been chronically ill (mean 5.1 years, range .8 to 16 years), although their mean \pm SD age was only 28.5 ± 8.6 . All patients had been treated previously with neuroleptic medication; for 21 patients the medication had been stopped three to four weeks prior to the lumbar puncture. Three additional patients could not be kept drug-free and were studied while stabilized on neuroleptic medication.

A group of 15 neurological patients, who had spinal fluid collected in an identical manner, were used as a reference group. The neurological patients included four with genetic muscular dystrophy, three with cerebellar degeneration, two with glycogen storage diseases, three with seizure disorders, and four with undiagnosed neurological complaints. CT scans were not available for the neurological controls.

CSF 5-HIAA concentrations were quantified with electrochemical detection, reversed phase, liquid chromatography.

Computed tomographic scans were performed at NIH in Bethesda, Maryland, with a 160 x 160 matrix head scanner (EMI 1010). All patients whose ventricular/brain ratios were at least two SD's greater than that of the mean for a control population (8.4%) were placed in the abnormal CT group (nine patients). The primary comparisons are within the schizophrenic patients because of limited available data on the neurological controls, although the CSF 5-HIAA in the three groups were compared with a one-way ANOVA.

There are no differences in CSF 5-HIAA concentrations between the 24 schizophrenic patients and the 16 neurological controls, 6.4 versus 6.7 ng/ml, respectively (Fisher Behrens t -test; $t=1.5$, ns). These values on the average, however, were much (about 70%) lower than those usually found with this assay, probably because they had been previously thawed. There are no differences in CSF 5-HIAA between the medication-free schizophrenic patients and the schizophrenic patients on neuroleptics ($t=1.3$, ns). The nine schizophrenic patients with abnormal cerebral ventricles had much lower CSF 5-HIAA than the 15 with normal ventricles, (2.9 ng/ml compared with 8.5 ng/ml ($t= 2.53$, $p<.03$). None of the nine schizophrenics with abnormal ventricles had 5-HIAA concentrations above the group median compared with seven of the 13 schizophrenics with normal ventricles (Fisher's Exact

Probability test, $p=.02$) and 13 of the 16 neurological controls (Fisher Exact Probability test for three groups, $p=.0002$). The overall ANOVA between the two schizophrenic groups and the neurological control group was significant ($df(2,24) = 10.01$, $p < 0.001$, Welsch approximation).

Within the schizophrenic patients there were no racial differences ($t = -.54$) or gender differences ($t(22) = 1.07$). The schizophrenics with enlarged ventricles are 7.9 years older than those with normal ventricles ($t=2.15$, $p < .05$), although the age range for the two groups is identical (21 to 44). The schizophrenics with enlarged ventricles were hospitalized and treated for the same length of time as the normal ventricle schizophrenics ($t=.57$, ns). The overall correlation between age and CSF 5-HIAA concentration is nonsignificant ($r=-.17$) as well as the correlation between length of hospitalization and CSF 5-HIAA concentration ($r=-.02$).

Three of the normal ventricle schizophrenics had higher 5-HIAA than any of the other subjects. We could find no distinguishing clinical characteristics of these three patients. Similarly, two of the abnormal ventricle schizophrenics had lower 5-HIAA than any of the other subjects. They as well are clinically indistinguishable from the other schizophrenic patients.

E. Apomorphine

Neuroleptics have proved to be the most effective treatment for schizophrenia, and also a valuable tool for research into the biochemistry of the disorder. Yet, there is a substantial proportion of chronic schizophrenic patients that do not respond to neuroleptics. Also, long-term treatment with neuroleptics is associated with a risk of inducing side effects such as a persistent tardive dyskinesia. Hence, there has been a continued search for treatments of schizophrenia with other drugs, instead of or in addition to neuroleptics. Among such therapeutic strategies is the use of low doses of apomorphine. It has been suggested that apomorphine, a direct but partial dopamine agonist, stimulates the inhibitory dopamine autoreceptors when administered in small doses. The consequent reduction of dopaminergic activity is postulated to be responsible for the putative antipsychotic effects of the drug. In this study, we assessed the effects of low-dose apomorphine on behavioral and neuroendocrine measures in schizophrenic patients with normal and abnormal CT scans.

The study sample consisted of 12 chronic schizophrenic patients. They were considered to be nonresponsive to "conventional" treatments for schizophrenia. There were nine male and three female patients, ranging in age from 21 to 44 years. Eight patients were white and four were black.

A "blind" planimetric evaluation of all the patients' CT scans showed normal scans in six patients, and abnormal scans in six. The latter group included one patient with cortical atrophy, and five with large ventricles, i.e., with a ventricle brain ratio greater than 8.4.

Each patient received subcutaneous injections of apomorphine (0.01 mg/kg) and saline on two different days, separated by at least 48 hours. The order of administration of the two injections was determined randomly for every subject. Blood samples were withdrawn through a heparin lock every 30 minutes over a two-hour period, for determining plasma growth hormone and prolactin concentrations. Clinical status was assessed in a double-blind manner, by two raters, using a modified version of the Brief Psychiatric Rating Scale (BPRS) every 30 minutes, immediately following each blood collection.

Eight of the patients were receiving stable doses of neuroleptics for at least a month prior to the study, while the other four were neuroleptic-free for a similar period. Two of the latter subjects were later retested with apomorphine after they had been on stable doses of neuroleptics for a minimum of one month. Five patients were tested with multiple doses of apomorphine (0.005, 0.01 and 0.04 mg/kg) on separate days. For prolactin and growth hormone measurements, plasma was removed and stored at -70°C until radioimmunoassays were done using the double antibody technique. The biochemical assays were performed "blind".

We found that of the 12 patients, two had a marked hypotensive reaction to apomorphine, (drop of more than 30 mm Hg in diastolic pressure), and could not be rated on the BPRS accurately. They were, therefore, excluded from further analysis of the BPRS data. We used means of the two raters' values for analyzing the BPRS scores. Interrater reliability for individual subscales ranged from .81 to .92 (intraclass correlation coefficient; $p < 0.001$).

Overall, there was no significant change in psychopathology with apomorphine or placebo as judged by change in total BPRS score. Analysis of values on BPRS subscales before and after apomorphine treatment showed a significant drop in scores for the syndromes of depression (from 4.2 ± 1.0 at baseline to 1.6 ± 0.7 at 60 minutes post-apomorphine; $p < 0.02$, matched pair t-test), and anxiety (from 3.8 ± 0.7 to 1.9 ± 0.5 at 60 minutes post apomorphine; $p < 0.01$, matched pair t-test). With placebo, there was no significant change in any subscale.

Breakdown of the patients into those with normal and abnormal CT scans revealed that both the patients who developed hypotension with apomorphine had normal scans. Defining improvement or worsening as greater than 25% reduction or increase, respectively, in placebo-corrected total BPRS score, we found that three of the normal CT scan patients worsened and one improved, while two abnormal CT scan patients improved and four were unchanged. This difference in response to apomorphine (Table 1) was significant at $p < 0.05$ (Fisher's Exact Probability test).

Table 1
CLINICAL RESPONSE TO APOMORPHINE

| CT Scan | Number of Patients | | |
|-----------|-----------------------|-----------|--------------------|
| | Improved ^a | Unchanged | Worse ^a |
| Normal | 1 | 0 | 3 |
| Abnormal* | 2 | 4 | 0 |
| | | | Total |
| | | | 4 ^b |
| | | | 6 |

*Difference between normal and abnormal CT scan groups significant at $p < 0.05$ (Fisher's Exact Probability Test).

^aImprovement or worsening was defined as 25% reduction or increase, respectively, in the total BPRS scores.

^bTwo other patients developed marked hypotension with apomorphine and could not be rated accurately on the BPRS.

Computing the placebo-corrected BPRS scores for subscales, abnormal CT scan patients had significant improvement with apomorphine on anxiety syndrome ($p < 0.02$), and a trend toward improvement on the syndrome of thought disorder and hostility/paranoia ($p < 0.1$, matched-pair t -test). There was no significant difference in response of the five individual patients to 0.005, 0.01 and 0.04 mg/kg apomorphine, as assessed by placebo corrected changes in total BPRS scores.

Of the patients tested while they were not receiving neuroleptics, two with normal CT scans developed hypotension, while two with abnormal CT scans were unaffected. The latter two patients were retested while on neuroleptics; there was no significant difference in their clinical responses on and off neuroleptics.

The only other side effects of apomorphine that we observed included mild nausea in two patients and sedation in three patients. These seemed to be unrelated either to CT scans or to concurrent neuroleptic administration. Plasma prolactin and growth hormone concentrations did not exhibit any consistent or significant changes following apomorphine.

Our results suggest that there was a significant difference between normal and abnormal CT scan patients in terms of their responsiveness to low-dose apomorphine. Whereas normal CT scan patients tended to worsen with 0.01 mg/kg apomorphine, those with abnormal CT scans had either no changes or some improvement. Since the two groups did not differ significantly from each other in age, gender, race, and clinical subtype or severity of schizophrenia, these variables did not explain the differential response to apomorphine. It is conceivable, however, that there were other differences between the two groups that we did not look for. Also, a possibility of differential pharmacokinetics of apomorphine cannot be excluded.

VII. Blinking

A. Animal Studies

In addition to a well-defined role in the control of movements, the cerebellum influences a variety of other behavioral and physiological functions. Among the organs affected by cerebellar function are the eyes. In rats, cerebellar lesions cause a temporary nystagmus. Cerebellar degeneration in man is associated with reduced saccadic eye movements and with the appearance of jerky eye movements and fixation nystagmus. One particular oculomotor sign that has not been studied in cerebellectomized animals is the frequency of spontaneous blinks. Therefore, the purpose of the present study was to evaluate the effect of cerebellar lesions on spontaneous eye-blink rates of rats.

Fifteen Sprague-Dawley albino rats weighing 225-250 g were subjected to total cerebellectomy by aspiration under chloral hydrate anesthesia. Nine animals survived the initial postoperative period and gained weight unassisted. Nine unoperated rats of the same age served as controls. There was a 10-month recovery period, after which observations were conducted once per week for six weeks in 12-inch diam clear Polycarbonate chambers. After 15-min adaptation to the chambers, each animal was observed for a total of six min in three 2-min epochs. A complete closure of either or both eyes was counted as one blink. The intraclass correlation coefficient for pairs of simultaneous observations by two observers was 0.95, $F(8,9) = 48.16$, $p < 0.001$. Observations of the animals' motor behavior were also recorded. Eight of the animals (one rat died) were then sacrificed and perfused with

phosphate-buffered formalin, and the brains sectioned at 80 μ m and stained with cresyl violet and by Page's solachrome cyanin method. The extent of the lesions was then determined by planimetric measurements of camera lucida drawings from sections corresponding to the planes located 2.4, 2.8, 3.2, and 3.6 mm caudal to the auditory meatus. The extent of the lesions was expressed as a percentage of the cerebellum in two dimensions remaining intact in each of the four planes.

There was a significant elevation of the overall mean (\pm SEM) blink rate of the cerebellectomized rats ($7.15 \pm 1.17/\text{min}$) as compared with the controls ($1.95 \pm 0.34/\text{min}$). Only one cerebellectomized rat had a blink rate ($3.17/\text{min}$) below that of the highest control blink rate ($3.28/\text{min}$). A two-way analysis of variance with one repeated measure showed the effect of cerebellectomy to be significant, $F(1,16) = 14.65$, $p < 0.002$. There was also a significant tendency for the blink rates of both cerebellectomized and control rats to decrease over the course of repeated observations $F(5,80) = 4.50$, $p < 0.002$. Interaction effects were not significant, $F(5,80) = 0.52$, $p = 0.236$.

Histological examination revealed extensive lesions with the deep nuclei and the vermis completely destroyed in all eight animals. Small portions of the lateral extremities of the cerebellar cortex were found to be intact in most of the animals. There was also slight damage to the ventral cochlear nucleus in six of the eight animals.

The three animals having the most complete lesions (Rat #'s 1, 4, 12) were found to have the lowest blink rates within the cerebellectomized animals, while the five animals that had a significant amount of cerebellar cortex remaining intact had relatively high blink rates. This tendency was particularly evident for the section corresponding to 2.8 mm caudal to the auditory meatus from Pellegrino and Cushman (1967); in this plane the percentage of the cerebellum remaining intact was positively correlated with blink rates (Pearson $r = 0.826$, $p = 0.012$). For the overall mean percentage of the cerebellum that remained intact (taken by averaging the percentage of the cerebellum remaining intact in each of the four planes) there was a slight, but not significant, correlation with blink rates ($r = 0.522$, $p = 0.183$). Blink rates were not correlated with the amount of damage to the ventral cochlear nucleus ($r = 0.358$, $p = 0.387$).

The histological findings suggest that the elevations in blinking may have been due to damage to a part of the cerebellum that was completely destroyed in all of the animals, such as midline structures or the deep nuclei. The presence of a significant amount of intact cerebellar cortex was associated with further increases in blinking, while blinking was increased only moderately in animals with complete lesions. Therefore, one area of the cerebellum may inhibit spontaneous blinking, while parts of the lateral cerebellar cortex might facilitate blinking. An analogous phenomenon is that movements can be either facilitated or suppressed by stimulation of various cerebellar areas. We are currently investigating the possibility that damage to a specific, as yet undefined, area of the cerebellum is responsible for the evaluations in blinking that are seen in totally cerebellectomized rats.

B. Human Studies

1. MAO

The major focus of research involving platelet monoamine oxidase (MAO) activity in the schizophrenic syndrome has been the role of lowered enzyme activity in this condition. This had led to neglect of another potentially interesting area of research of the relation-

ship between brain monoamine activity, particularly dopamine, and platelet MAO activity. It has been found that a positive correlation between plasma prolactin concentration and platelet MAO activity exists. Since dopamine released from the hypothalamus inhibits prolactin release it can be argued that platelet MAO activity may be inversely related to central dopamine activity.

Evidence suggests that eye blink rates may provide another means to test the relationship between platelet MAO activity and central dopamine activity. Since it appears that platelet MAO activity may inversely correlate with central dopaminergic activity and blinks increase with increasing central dopaminergic stimulation, we would expect an inverse correlation between these two variables. Such a relationship has been demonstrated in a study in which both blink rate and MAO activity were derived without regard to medication status. It now appears that neuroleptics reduce both platelet MAO and blink rates. To further investigate this relationship we studied 27 chronic schizophrenic inpatients (21 males, 6 females) and 36 normal volunteers.

Since speech increases blinking, all subjects were counted while they participated in clinical interviews. During medication free periods, (mean duration \pm SD = 7 ± 3 weeks, range 2-12 weeks) blinks were counted weekly. The medication free blink rate is the mean of these counts.

Blood was drawn from all subjects and put into plastic tubes containing EDTA-citrate. Platelets prepared by the Corash method were analyzed for MAO activity by modification of the Wurtman and Axelrod method. The drug free platelet MAO activity was the mean activity of the weekly platelet MAO determinations after the second drug free week. For normal controls the procedures were the same except that blink rates and platelet MAO values were usually determined once.

All patients were observed for the presence of abnormal movements. TD was diagnosed using specific criteria. Usually, if a persistent dyskinetic movement with a severity 2+ or more in any of the seven motor groups described in the Abnormal Involuntary Movement Scale (AIMS) was observed, the patient was counted as having TD. There were seven patients with TD, four males and three females (mean age 33 ± 8 years).

Table 1 shows the mean values of blink rates and MAO activity in each of the subject groups and the correlations between platelet MAO and blink rate. There is a significant correlation for all patients ($r_s = -.35$, $p < .05$) which is stronger when patients with TD are excluded ($r_s = -.47$, $p < .025$). There is no significant correlation in normal controls and patients with TD.

Table 1

MEAN MEDICATION-FREE BLINK RATE AND PLATELET MAO ACTIVITY IN THEIR CORRELATION

| | Blink Rate (Blinks/Minute) | Platelet MAO (nM/mg/hour) | Correlation Between Blink Rate and Platelet MAO Activity |
|--------------|-------------------------------|------------------------------|---|
| Controls | 22 ± 13 | 21.6 ± 5.1 | -.08 |
| All Patients | $28 \pm 17^{*a}$ | 22.8 ± 6.3 | -.35 ^{*b} |

Subgroups:

| | | | |
|--------------------------------|-------------|----------------|--------|
| Patients without Dyskinesia | 27 \pm 18 | 23.5 \pm 5.2 | -.47** |
| Patients with Dyskinesia | 31 \pm 12 | 20.8 \pm 8.9 | -.07 |

*p < .05

**p < .025

^aT-test compared to control group. One tailed assumption.^bSpearman's r (r_s), one-tailed assumption.

2. Dopamine

Reduced central dopamine (DA) and spontaneous blink rates in Parkinson's disease and the increase of DA activity and blink rates in schizophrenia and Tourettes Syndrome suggest that spontaneous blink rates may, also, be correlated to central dopamine activity. Furthermore, apomorphine, a dopamine agonist, increases blink rates while haloperidol decreases blinking in monkeys. If blink rates are determined by central DA activity, than an important question to ask is where is the anatomical location of blink activity? While the effect in Parkinson's disease suggests that the basal ganglia may be an appropriate site, a relationship found between psychotic symptoms and spontaneous blink rates in schizophrenic patients suggest that blinks may be mediated by the same dopaminergic pathways. The purpose of this experiment is to determine which central DA system regulates blink rates.

Two observers, both blind to the treatment conditions, observed primate behavior while positioned one meter away from the cage, with their eyes level with the bottom of the cage. The observer recorded two classes of behavior: blinking and general behavior. The observers counted each individual blink that occurred during a 15-second period. The time interval was determined by an electric timer which produces a low pitched buzz at the end of the elapsed recording period. The general behavior was also recorded for a 15-second period using the method just described. There were 27 objectively defined categories of behavior that were scored either present or absent. Coefficients of correlation of interrater reliability were determined, and actual experimentation did not commence until the rating is .90 or above.

The procedure for any single injection involved two pretreatment observation sessions at 30 and 15 minutes (+30, +15) before injection. There were four recording sessions following the injection (time 0) at 15, 30, 45 and 60 minutes (+15, +30, +45, +60). During each session, blinking was counted for 15 seconds, general behavior for 15 seconds, and a 15-second period for recording data on a scoring sheet. A total of six 45-second cycles was carried out during each observation session.

For each observation session, blinking counts were averaged from the six 45-second cycles to determine the count for the 4.5 minute observation session, and were compared to the totals for the other observation sessions.

General behavior was broken down into non-stereotyped; stereotyped; and general activity. These were listed on a rating scale, followed by detailed analysis of each behavioral

classification. General behaviors, were rated on a scale of 0-4 according to estimated energy expenditure.

Monkeys were anesthetized with Ketamine HCl (.5 ml IM) initially, with subsequent lower doses to maintain anesthesia during surgery. Using the stereotaxic atlas for Rhesus by Snider and Lee, a stainless steel 23 gauge canulae were placed in the nucleus accumbens. Permanent recording electrodes for measurement of eye blinks were implanted above and below each eye. Following recovery, the animal was placed in a metabolic chair for recording.

Two weeks following surgery, baseline blink rates were recorded for two hours before injecting normal saline into the canula. Dopamine was administered progressively until an increase in blinking was seen, or until it was apparent that no effect was present.

Following DA injections, haloperidol was administered through the canula to determine whether or not it decreased blink rates. Finally, DA was given at a blink increasing dose, followed by haloperidol to determine whether the later blocks the effects of DA. The effect of drugs on blinking was measured for two hours post-drug administration.

The final step of the experiment involved the administration of 6-OHDA to see whether or not blinks were abolished. If these injections failed to alter blinking, the animal was canulated at a site in the basal ganglion. Subsequent animals were then canulated only at sites demonstrated to be associated with blinking and alteration by dopaminergic drugs. We are now analyzing these data.

C. Pharmacological Studies

Recently, we have found that blink rates correlate inversely with platelet monoamine oxidase (MAO) activity in schizophrenic patients. To investigate this further, we have been studying pharmacological manipulations in monkeys to more clearly elucidate the roles of MAO in blinking.

Four male juvenile *Macaca mulatta* monkeys weighing between 5 and 7 kg were individually housed in cages which did not allow visual contact between animals. All animals were food-deprived 24-hr prior to testing. Water was available ad lib. All experiments were conducted in the individual animals' home cage.

Two observers simultaneously counted the number of blinks that occurred during 15-sec periods as described below. General behaviors were scored with particular attention being paid to two oral-facial movements: lipsmack-grimace and chew-gnashing. The latter is an extreme oral movement with a sharp opening and closing of the jaws and lateral movements of the mandible associated with the grinding of teeth, producing audible squeaking or percussive sounds.

All trials consisted of six sessions, 15 min apart. Each of these sessions was composed of six 15-sec counting periods for blink rates, alternating with six 15-sec counting periods for behaviors. Blinking scores for each session were the sum of the six 15-sec observation periods (results are reported as blinks/90 sec). Behaviors were scored as either absent or present and summed for the six counting periods. For the two oral-facial movements, the maximum score was 12 (both behaviors present during each observation session).

For most trials, the first two sessions served as base-line controls. The exception to this was that three base-line sessions were obtained in animals treated with haloperidol. Injections were administered following the second session, with a 15-min waiting period before the third session was begun.

On Day 1, saline (0.1 mg/kg) was given intravenously to obtain base-line data. On Day 3, an intramuscular dose of B-phenylethylamine (PEA, 75 mg/kg) was administered and the animals rated. On Day 6, injections of pargyline (0.5 mg/kg, iv) were begun and continued for nine days. On Day 15, PEA was again administered at a dose of 10 mg/kg.

A second study was begun after a 14-day drug-free interval.

Apomorphine (0.02 mg/kg) was administered subcutaneously on Day 1 with a higher dose (0.36 mg/kg) administered the following day. Each animal was then treated with haloperidol (1 mg/kg, im) for four days. On the fourth day of haloperidol treatment the animals again received apomorphine (0.36 mg/kg).

Phenylethylamine given to animals who received chronic pargyline treatment significantly increased blink rates. This effect plateaued at Session 4 (30 min after injection) and was maintained through the last session. A parallel increase in mean \pm SD mouth movement scores from 2.8 ± 1.7 to 12 ± 0 also occurred in the animals receiving this treatment.

While it appears from Table 1 that chronic pargyline may increase blinking (18.2 ± 5.3 blinks/90 sec) compared to animals receiving saline (14.5 ± 6.5 blinks/90 sec) or on the first day of pargyline treatment (12.0 ± 1.4 blinks/90 sec), the small number of animals prevents a statistical comparison.

Table 1
ACUTE EFFECTS OF PEA AND PARGYLINE ON BLINKING
(MEAN BLINKS/90 SEC)

| | Session No. | | | | | | |
|--|-------------|------|------|------|------|------|-------------------------|
| Treatment | 1 | 2 | 3 | 4 | 5 | 6 | χ^2_a |
| Saline | 13.5 | 16.7 | 15.3 | 15.5 | 12 | 10 | 9.07 |
| PEA only 75 mg/kg | 15.5 | 10.3 | 10.8 | 13.5 | 9.3 | 7.5 | 4.57 |
| Pargyline, Day 6 | 14 | 12.3 | 13.3 | 10.3 | 11.5 | 11.7 | 6.32 |
| Pargyline, Day 14 | 17.5 | 21.3 | 17.3 | 15.8 | 19.5 | 15.8 | 8.07 |
| PEA 10 mg/kg in paryglyne-treated animals (Day 15) | 12 | 15.5 | 31 | 42.8 | 42.7 | 38 | 13.71 ($p < 0.02$) |

^aFor Friedman's two-way analysis of variance

laboratory's previous finding of enlarged cerebral ventricles in the brains of some schizophrenic patients, we are now correlating ventricular size with a wide array of other measures. These include poor premorbid history, antipsychotic drug response, spontaneous eye blink rates, age, duration of illness and at autopsy, evidence of catecholamine production in the brain. Our CT scan work has been repeatedly cited in the literature and multiple laboratories are beginning to use ventricular size as a measure for subgrouping schizophrenic patients.

Our research into possible etiological factors has also produced innovative histological findings. It appears, in preliminary work, that we have found evidence of cytomegalovirus in several limbic region sections of the brains of some schizophrenic patients. These findings provide tentative support for the viral hypothesis of schizophrenia, which has been only indirectly supported, up until now, by epidemiological studies.

And finally, we are continually reassessing all of our schizophrenia research with an eye to developing a new method of subtyping schizophrenic patients. To date, patients have been divided according to the overt behavioral manifestations of their individual psychoses. We are beginning to move towards a new typology, one, perhaps, better organized along physiological, neuroanatomical and pharmacologically responsive dimensions. By developing a new classification system, it is hoped that new perspectives on research into etiologies, prevention and treatments will result.

Proposed Course

We plan to continue examining the schizophrenia syndrome from a multi-disciplinary perspective. To this end, we will continue to search biochemically for markers through the elucidation of abnormalities in the production and function of catecholamines, enzymes and hormones. We plan to continue refining our ability to assess neuroanatomical and metabolic findings derived from such technological innovations as computerized axial tomography (CT) scans, cerebral blood flow and positron emission tomography scans. We plan to continue our etiological investigations into a possible viral component of the disorder. And in a manner that coherently connects this diverse body of research, we will continue to work towards a more productive typology of the schizophrenia syndrome.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01337-11 SMRA |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies on Drugs of Abuse | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R.J. Wyatt, Chief, Adult Psychiatry Branch, SMR; J.C. Gillin, Deputy Chief, Adult Psychiatry Branch, SMR; E. Parker, Psychologist, NIAA; W.B. Mendelson, T.P. Bridge, R.J. Wagner, J.E. Kleinman, L.E. DeLisi, J.M. Morihisa, Staff Psychiatrist, Adult Psychiatry Branch, SMR; W.J. Free, Staff Fellow, Adult Psychiatry Branch, SMR; F. Karoum, Chemist, Adult Psychiatry Branch, SMR. OTHER: H. Weingartner, Psychiatrist, DCBR S. Hashtroudi, George Washington University | | |
| COOPERATING UNITS (if any) Division of Research, National Institute of Drug Abuse, Adult Psychiatry Branch, National Institute of Mental Health and George Washington University. | | |
| LAB/BRANCH Adult Psychiatry Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 6 | PROFESSIONAL: 4 | OTHER: 2 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The long range purpose of this project continues to be investigation of the mechanisms of action of drugs of abuse and the effects of alcohol consumption. Our hope is that increased understanding of drug effects can help us further differentiate normal mental function and mental illness. The report that follows brief summaries of research initiated and the update on continuing work in our laboratory during the 1981-1982 reporting period. The summaries have been broken down into categories. | | |

Objectives:

The objectives of the Substance Abuse Program in the Adult Psychiatry Branch is to formulate and investigate hypotheses concerned with the nature and action of pharmacological agents that are either classified as or can become, through misuse, drugs of abuse. Through research examining the mechanisms of these substances, we hope to better understand their use and the effects of their abuse.

Alcohol

A. Monoamine Oxidase (MAO)

In our search to better understand biochemical mechanisms and their relationship to alcohol consumption, we studied approximately 200 area university students (males, ages 18-30), examining MAO and plasma DBH activity in relation to alcohol consumption. In this population, MAO activity was significantly correlated with disinhibition on the Zucherman Scale and extraversion on the Eysenk Personality Scale. MAO activity was significantly lower in those with a family history of alcoholism compared to those subjects without such a family history.

MAO activity was positively correlated with time from last drink and frequency of alcohol use. Subjects with a positive family history were matched to subjects with a negative family history on multiple measures of alcohol use. No differences between the two groups were found, nor were there any correlations for plasma DBH activity.

B. Memory

The processing-deficit hypothesis has received some support from studies on Korsakoff Syndrome, the permanent amnesia associated with years of severe alcohol abuse. There is also some evidence that a similar mechanism may be relevant for understanding the effect of acute alcohol intoxication on memory. It has been found that presentation of context words reduced the detrimental effect of alcohol on a recognition task, thereby providing evidence in support of processing failure under alcohol.

The first question we addressed was whether intoxicated subjects can use subtle elaborators that facilitate normal memory. It has been suggested that effective elaborators specify the potential significance and relevance of target words, rather than simply putting the words in the context of semantically congruous knowledge.

The second question addressed was whether acute alcohol amnesia is attenuated when subjects are forced to engage in elaborative processing. This possibility is particularly interesting in light of recent findings on the effect of self-generated versus experimenter-provided events on memory. The very act of generation can enhance memory either for the generated event or, in some situations, for the stimulus on which the generation operation is performed. This memory enhancement might be independent of other semantic or interpretive operations.

The third and related issue we investigated concerns the quality of semantic structures activated during intoxication. The subject-generation condition is particularly important because it permits an examination of the quality of elaborators produced by sober and

intoxicated subjects. In sober subjects, the effectiveness of self-generated elaborators for recall may depend on the precision of these elaborators; self-generated elaborators are effective only if they clarify the meaning or the relevance of target words relative to the sentences in which they are embedded.

In our experiment, subjects were either sober or intoxicated. There were four memory conditions: no elaborators, experimenter-provided precise elaborators, experimenter-provided imprecise elaborators, and subject-generated elaborators. Cued recall of target words was tested with base sentences (with the target word missing) as cues.

All subjects were tested individually and were given instructions about the memory test before ingestion of their drinks. After the drinking and absorption period, subjects were told that they would hear a list of sentences on the tape recorder and that they would be given a memory test following presentation of the list. In addition, to insure attention to the sentences, subjects in the three experimenter-provided elaboration conditions rated the comprehensibility of each sentence on a scale of 1 to 5. Subjects in the self-generated groups were told to write a short phrase continuing each sentence. Two sentences were used as examples to illustrate the rating or the generation tasks. Following the presentation of each sentence, 15 seconds were allowed for performance of the rating and the generation tasks.

After acquisition, subjects were asked to count backwards by threes for 1 minute. They then heard the 12 base sentences with blank words, and they had 7 seconds to recall each target word. A breath reading was taken when recall ended.

We found that intoxicated subjects could not utilize normally effective but subtle elaborators. Second, self-generated elaborators improved recall of target words for both sober and intoxicated subjects, but they did not eliminate or reduce the negative effect of alcohol. Finally, a direct assessment of the quality of generated elaborators revealed that the elaborators produced by intoxicated subjects were strikingly similar to those generated by sober subjects.

The results clearly show that there is a marked deficit in processing semantic information under alcohol. In contrast with sober subjects who showed the expected memory enhancement with precise elaborators, intoxicated subjects were unable to take advantage of these elaborators to improve their recall of the base sentences. This deficiency in using precision is a reliable finding: It was replicated in two different conditions. When the experimenter provided precise elaborators and when subjects generated their own precise elaborators, recall of sober subjects increased markedly but recall of intoxicated subjects did not. This work demonstrates, for the first time, that a highly effective semantic encoding manipulation for normal subjects magnifies the memory deficit associated with acute alcohol intoxication. The precision dimension offers a powerful tool for analyzing alcohol-induced amnesia.

C. Marijuana

Alcohol is a drug with powerful amnesic effects. In humans, high acute doses of alcohol can produce blackouts for events experienced during a drinking episode. More common is the less severe amnesia that occurs in both alcoholics and social drinkers.

The formation of new memories is significantly disrupted by acute doses of alcohol that leave intact the capacity to retrieve old memories. For example, when subjects learn-

ed a task while sober and were tested for recall one week later under either sober or alcohol conditions, those subjects who had ingested alcohol were just as fast and accurate as non-drugged subjects in retrieving what they had learned the week before. Their new learning, however, was significantly impaired.

It has been suggested that alcohol may interfere with memory trace consolidation. According to this hypothesis, alcohol interferes with those processes underlying the solidification of the memory trace. Consolidation is studied primarily in animal research on the psychobiology of memory by treating animals shortly after training during the time that consolidation is thought to occur. Since both acquisition (learning) and retrieval (remembering) take place in the untreated state, changes in retention are thought to reflect alterations in trace consolidation. Following this approach, we have conducted two experiments in which subjects ingested alcohol immediately after learning and were tested for recall later in the nondrugged state.

The first experiment examined the post-acquisition effects of alcohol and marijuana on human memory. Only alcohol had a significant effect and it enhanced memory. It seemed paradoxical that a drug which normally depresses memory could produce facilitation when introduced after the learning experience. A second experiment was therefore conducted which confirmed and extended the original finding.

In the first experiment, subjects were 16 normal male volunteers who were moderate users of alcohol and marijuana, and had a mean age of 24 years. They were tested individually on two days separated by one week. Each test day involved a nondrug baseline session in the morning and a drug session in the afternoon. Drug order was counterbalanced across days.

At the onset of each session, subjects studied 10 scenic slides displayed for 5s each with a 1-s interstimulus interval. Recognition was tested at the end of each session approximately 3 h later. To test recognition, 10 pairs of slides were displayed one pair at a time. In each pair of slides there was one that had been studied at the outset of that particular session and one that was new but very similar to the original. The subject selected the slide he believed he had seen before. The recognition test was self-paced. A different set of slides was used in each session and four equally difficult sets were counterbalanced with conditions. Further details about this task are reported elsewhere in an experiment which found dose-dependent impairment when subjects ingested alcohol (0.5 or 1.0 mg/kg) before studying the pictures.

In the drug sessions, immediately after studying the slides, subjects were given either alcohol (1 m/kg) or marijuana (15 THC). The alcohol was administered as one part USP ethanol and three parts fruit juice over 30 min and produced a mean peak blood alcohol level (BAL) of 0.08 g/100 ml 55 min after initiation of drinking. The marijuana was smoked over 10 min resulting in a 42% change in pulse 15 min after initiation of smoking. In both the drug and nondrug sessions subjects performed a verbal memory task that will be discussed in a separate communication.

Drug performance was compared to nondrug baseline for the appropriate day using the two-tailed Sign Test.

In the second experiment, subjects were 72 males, light to moderate drinkers as measured by Cahalan's quantity-frequency-variability classification. They were randomly assigned either to an alcohol or to a placebo group. The mean age for both groups was 23

years and mean vocabulary abilities were equivalent for the groups as estimated by the Shipley-Hartford Institute of Living Scale.

On the first morning immediately before the drinks were consumed, subjects were presented with a sorting task, ostensibly to assist us in selecting materials for the next experiment. Thirty words were sorted into five categories. The use of an incidental learning task was designed to minimize rehearsal.

Upon completion of the sorting task subjects consumed either placebo or alcohol drinks in a 40-min period. A dose of 1 ml/kg alcohol was administered in equal volumes of vodka and masking solution. The placebo drinks contained water in place of vodka in glasses swabbed with vodka. Subjects then engaged in several other learning tasks to control the nature of subsequent cognitive activities.

The next day subjects returned to the laboratory to describe their experiences from the previous day. After several questions they were given an unexpected recall test for the words they had sorted the day before. Two minutes of free recall were followed by a cued-recall test in which the category names were provided. Analysis of variance was performed on three recall measures: total number of words correctly recalled, number of categories recalled, and number of words per category. Alcohol condition was a between-subjects factor and free vs cued recall was a within-subjects factor. Data for one subject were excluded because he returned to the laboratory 5 h later.

With the first experimental group, when subjects had ingested alcohol immediately after studying the pictures, their recognition performance at the end of the session was significantly better compared to nondrug baseline. In the alcohol condition 7/16 subjects correctly recognized all 10 pictures; whereas, only two subjects had a score of 10 correct in the nondrug baseline session. In contrast with alcohol, smoking marijuana after original learning did not significantly alter recognition memory. Recognition of pictures that had been studied immediately before drug administration was significantly better after alcohol than after marijuana.

Only alcohol retroactively enhanced memory for information acquired before drug ingestion, yet both alcohol and marijuana typically produce anterograde amnesia. The anterograde depression of memory for information learned under the influence of alcohol and marijuana was seen in this experiment. Free recall of words studied after drug ingestion dropped 28% and 14% under alcohol and marijuana respectively.

Results of the second experimental group showed that recall after 24 h was significantly better in those subjects who had received alcohol immediately after studying the words, the alcohol group recalled more words and more categories than the sober group, and about the same number of words per category. Providing category cues significantly increased recall; nevertheless, the alcohol group was still superior to the placebo group.

D. Preserved Memories

There appears to be a relative sparing of certain memory processes in amnesic patients. It has been reported that amnesic patients, deficient in the ability to recall or recognize recently presented words, perform equally to nonamnesic controls when their task is to identify fragmented words. The purpose of the present study was twofold: first, to compare the memory deficits in amnesic patients with the amnesic state associated with

alcohol intoxication, and second, to examine in some detail the memory processes which are sensitive or resistant to amnesia.

A total of 128 subjects viewed 29 unrelated high-frequency words which they were instructed to remember. They were then tested by free recall, simple yes-no recognition, or the fragmented-word identification method. Half of the subjects in each testing condition were in the placebo condition and the other half in the alcohol condition (dose of 1 ml/kg).

Our results show that as with Korsakoff patients, acutely intoxicated subjects were most impaired in the free recall condition. Intoxicated subjects were as good as sober subjects in identifying fragmented versions of previously seen target words. Thus, intoxicated subjects could identify already studied target words sooner (at a higher level of degradation) than distractor items which they had never seen before.

One apparent exception to the parallel between acute alcohol amnesia and Korsakoff's amnesia is seen in the simple recognition condition. Surprisingly, intoxicated subjects were just as good as sober subjects when they were simply asked to judge whether a word was one they had seen before. It appears that the processing of basic familiarity information can proceed normally under alcohol. However, when subjects have to resort to search-based processes rather than familiarity alone, memory impairments begin to emerge. In modified recognition condition, where subjects were asked to recognize a word as old or new at the point they had identified its fragmented cue, intoxicated subjects exhibited recognition failure. When subjects were taken out of an elementary familiarity mode, memory failure set in. This is consistent with the finding that Korsakoff patients are impaired on recognition when they cannot rely on simple familiarity.

Under alcohol, certain memory processes are resistant to the effects of alcohol. In particular, subjects appear to be able to acquire event representations which are sufficient for making simple familiarity judgements or for identifying degraded word fragments. In contrast, other memory processes are particularly sensitive to alcohol's acute amnesic effects. We suggest that intoxicated subjects have deficits in the formation of event representations which permit successful search-based retrieval, such as free recall.

PCP

In recent years, widespread abuse of the drug phencyclidine (PCP) has caused an increasing number of hospitalizations due to severe and sometimes violent toxic reactions. These adverse reactions frequently resemble psychotic episodes involving violence, agitation, and bizarre behavior. Such reactions have been reported to last for as long as several weeks. Therefore, some form of pharmacological treatment frequently is indicated.

There is, however, some controversy regarding the preferred form of treatment, some researchers recommend benzodiazepines while others prefer neuroleptics. In animals, a number of studies agree that at least some of the effects of PCP can be blocked by neuroleptics. Little data exist, however, comparing various neuroleptics in their PCP-blocking efficacy. Several studies have reported that both haloperidol and pimozide are effective blockers of various effects of PCP. There has been one report, though, demonstrating that pimozide is somewhat less effective than haloperidol in blocking PCP-induced rotational behavior. Haloperidol has been found, also, to be less effective than chlorpromazine and clozapine in blocking PCP-induced locomotor stimulation in mice. These findings suggest that there may be substantial differences among neuroleptics in their ability to block the effects of PCP.

To begin differentiating among various neuroleptics, a total of 450 adult female Swiss-Webster mice were housed in groups of 4-8 and allowed free access to food and water. Animals were housed on a 12-h light-dark cycle. Each mouse was used only once.

Phencyclidine HCl (1-(1-phenylcyclohexyl) piperidine HCl), was dissolved in normal saline in concentrations such that a dose of 5.0 mg/kg in a volume of 10 mg/kg was administered by IP injection.

Other drugs used were dissolved in saline, except in a few cases where it was necessary to employ saline slightly acidified with HCl as a vehicle. Drug solutions were made just prior to use, and administered IP in a volume of 10 mg/kg. Dosages were calculated as the salts.

The behavioral activity of each animal was measured in cylindrical Polystyrene jars with a 16.3-cm inside diameter by 23 cm high, which were placed on one of eight similar activity meter units, using the horizontal photocell banks only. The apparatus was illuminated from above by 50 watt red lights covered by safelight filters.

Each mouse was given either a neuroleptic drug or vehicle, and the activity of each mouse was then measured for 30 min. The activity meters were then turned off for 5 min, during which each mouse was given 5 mg/kg of PCP IP. Some of the animals were given saline vehicle for this second injection. The activity of the animals was then measured for a second 30-min period.

Data Analysis

The data were collected every 10 minutes for the first and second 30-min period of the activity tests, and examined in terms of 1) total activity during the first 30-min period, a measure of the effects of the drugs in the absence of PCP, and 2) the ratio of the total activity during the second 30 min period to the total activity during the first 30-min period, which was considered to be a measure of the degree of stimulation produced by PCP (stimulation ratio).

Data were analyzed statistically by means of an one-way analysis of variance followed by Scheffe multiple comparisons. The null hypothesis was rejected at the 0.05 level.

The efficacy of the drugs was quantified in three ways: 1) the dose which decreased PCP stimulation ratios by 50%, using the vehicle stimulation ratio as a baseline, or the "PCP ED₅₀" 2) the dose which decreased general activity (prior to PCP administration) by 50%, or the "activity ED₅₀", and 3) the ratio of the PCP ED₅₀ to the activity ED₅₀, or the "relative efficacy". The ED₅₀'s were determined graphically, by inspection of the dose-response curves. These measures were correlated with other measures from the literature using Pearson correlation coefficients. Logarithmic transformations were used for dosage and concentration measures.

Results

PCP produced a pronounced stimulation of motor activity. The mean (\pm S.E.M.) stimulation ratio for the PCP-treated mice was $2.02 \pm .15$, as compared with a mean stimulation ratio of $0.52 \pm .04$ for animals treated with vehicle.

Most of the neuroleptics that were tested blocked this effect of PCP, in that they reduced the stimulation ratios as compared with mice treated with PCP alone. In addition, most of the drugs reduced the activity of the animals prior to administration of PCP (Table 1).

Table 1
Blockade of PCP-Induced Stimulation by Neuroleptics*

| Drug | Dose (mg/kg) | N | Activity Before PCP** | Stimulation Ratios*** |
|----------------------|-----------------|----|-----------------------------|-----------------------------|
| Vehicle | --- | 53 | 167 \pm 9.7 | 2.02 \pm .15 |
| Chlorpromazine HCl | 2.5 | 11 | 116 \pm 25.5 ^a | 1.16 \pm .28 ^a |
| | 5.0 | 14 | 79 \pm 13.7 ^e | 0.79 \pm .28 ^c |
| | 10.0 | 7 | 79 \pm 4.3 ^e | 0.18 \pm .05 ^f |
| | 20.0 | 6 | 23 \pm 3.6 ^g | 0.25 \pm .08 ^f |
| Clozapine | 2.5 | 8 | 70. \pm 10.1 ^f | 1.57 \pm .14 |
| | 5 | 8 | 49 \pm 8.9 ^f | 1.09 \pm .16 ^b |
| | 10 | 8 | 35 \pm 6.0 ^g | 0.70 \pm .20 ^e |
| Fluphenazine 2 HCl | 0.25 | 7 | 169 \pm 16.4 | 1.17 \pm .16 ^e |
| | 0.5 | 7 | 134 \pm 28.6 | 0.38 \pm .12 ^f |
| | 1.0 | 6 | 129 \pm 12.0 | 0.35 \pm .12 ^f |
| | 2.0 | 7 | 73 \pm 15.3 ^e | 0.27 \pm .07 ^e |
| Haloperidol | 0.5 | 12 | 105 \pm 19.3 | 1.28 \pm .27 ^a |
| | 1.0 | 10 | 53 \pm 8.9 ^g | 1.01 \pm .34 ^c |
| | 2.0 | 10 | 37 \pm 10.6 ^g | 1.14 \pm .33 ^b |
| Methiothepin Maleate | 0.156 | 9 | 210 \pm 19.6 | 1.59 \pm .27 |
| | 0.312 | 9 | 185 \pm 35.9 | 1.05 \pm .29 ^b |
| | 0.625 | 9 | 198 \pm 18.9 | 0.50 \pm .12 ^e |
| | 1.25 | 6 | 153 \pm 18.6 | 0.13 \pm .04 ^f |

| | | | | |
|------------------|------|----|-----------------------------|------------------------------|
| | 2.5 | 6 | 126 \pm 14.6 | 0.05 \pm .02 ^f |
| | 5.0 | 6 | 51 \pm 14.8 ^e | 0.86 \pm .70 ^c |
| Molindone HCl | 2.5 | 8 | 47 \pm 9.6 ^f | 7.11 \pm .78 ^g |
| | 5.0 | 10 | 115 \pm 42.4 ^a | 3.17 \pm .67 |
| | 10.0 | 10 | 33 \pm 5.8 ^f | 6.62 \pm 1.84 ^g |
| Pimozide | 0.25 | 9 | 125 \pm 27.5 | 4.82 \pm 1.18 ^g |
| | 0.5 | 9 | 179 \pm 16.1 | 2.42 \pm .29 |
| | 1.0 | 6 | 168 \pm 40.5 | 2.33 \pm .30 |
| | 2.0 | 24 | 207 \pm 26.0 | 1.03 \pm .14 ^a |
| Sulpiride | 10. | 7 | 137 \pm 15.8 | 1.84 \pm .33 |
| | 20. | 9 | 131 \pm 9.7 | 2.61 \pm .34 |
| | 40. | 8 | 132 \pm 9.0 | 1.84 \pm .30 |
| | 80. | 8 | 123 \pm 10.4 | 1.67 \pm .19 |
| Thioridazine HCl | 5.0 | 10 | 145 \pm 23.8 | 1.72 \pm .55 |
| | 10. | 10 | 55 \pm 9.7 ^f | 1.11 \pm .26 ^a |
| | 20. | 6 | 58 \pm 17.1 ^f | 0.22 \pm .10 ^f |
| Trifluoperazine | 1.0 | 8 | 117 \pm 13.4 ^a | 1.65 \pm .41 ^b |
| | 2.0 | 8 | 119 \pm 18.8 ^a | 0.95 \pm .40 ^f |
| | 4.0 | 8 | 101 \pm 20.9 ^c | 0.38 \pm .09 ^d |
| | 6.0 | 8 | 67 \pm 10.4 ^f | 0.75 \pm .22 ^e |
| | 8.0 | 9 | 57 \pm 12.2 ^f | 0.64 \pm .27 ^c |
| <hr/> | | | | |
| No PCP**** | --- | 49 | 166 \pm 9.5 | 0.52 \pm .04 |

*Data are shown as means \pm S.E.M.

**Activity before PCP, after administration of drug.

***Stimulation ratios are the ratio of activity during the 30 min after PCP administration to the activity before PCP administration.

****Animals received vehicle followed after 30 min by saline.

$a=p < 0.10$, $b=p < 0.05$, $c=p < 0.02$, $d=p < 0.01$, $e=p < 0.005$, $f=p < 0.001$, and $g=p < 0.0001$ as compared to vehicle (Scheffé tests performed following significant effects from one-way analysis of variance).

The three drugs that did not block PCP were molindone, pimozide, and sulpiride. To avoid the potential pitfall of missing a significant blocking effect of pimozide due to testing too few animals, or insufficiently high dosages, twenty-four mice were tested at a very large dosage (2.0 mg/kg). Even at these high dosages pimozide did not significantly decrease stimulation ratios. Lower dosages of pimozide, and all dosages of molindone that were tested actually tended to enhance the effect of PCP. Pimozide and sulpiride also did not decrease the activity of the animals prior to PCP administration. In addition, the blocking effect of haloperidol, although statistically significant was incomplete and was not dose-dependent (Table 1).

There were large differences between the various drugs in terms of their relative efficacy, i.e.; the PCP ED_{50} divided by the activity ED_{50} . The extremes were methiothepin, with a relative efficacy of 0.06, and clozapine, for which the relative efficacy was 2.3, i.e., 2.3 times the ED_{50} for activity was required to block PCP by 50%. According to this criterion, the effective drugs were ranked as methiothepin (relative efficacy = 0.06), fluphenazine (0.13), trifluoperazine (0.27), chlorpromazine (0.55), thioridazine (1.05), haloperidol (1.10), and clozapine (2.33), from most to least effective.

An attempt was made to relate the effectiveness of the various drugs to their potency in various binding studies and behavioral tests reported in the literature. The most striking finding was that the PCP ED_{50} was very strongly correlated with the efficacy of the drugs in blocking tryptamine-induced seizures (Pearson's $Rho = 0.95$; $p = 6 \times 10^{-4}$) and with their efficacy in inhibiting dopamine binding as evaluated by Burt *et al.* (1976) ($Rho = 0.94$, $p = 3 \times 10^{-4}$). In general, the efficacy of the drugs was related to several dopaminergic and serotonergic parameters.

Our data confirm a number of previous reports that behavioral effects of PCP in animals can be attenuated by neuroleptics. In general, the best PCP antagonists (methiothepin, fluphenazine, and trifluoperazine) are very potent clinically (on a mg/kg basis), while several of the less potent PCP antagonists (thioridazine, clozapine, molindone, and sulpiride) are less potent clinically. Chlorpromazine, however, was very effective, even though it is not very potent clinically. Pimozide and haloperidol, also, were relatively ineffective despite their great clinical potency. Pimozide was almost entirely ineffective, and the blockade of PCP that was produced by haloperidol was not dose-dependent and never reached 100%. This suggests that some property of neuroleptics other than their primary therapeutic action is involved in their ability to antagonize PCP-induced stimulation in mice.

The neuroleptics are thought to act clinically, in the treatment of schizophrenia, by blocking central dopamine receptors. Many neuroleptics, however, have substantial antiserotonergic activity as well. We found that the ability of neuroleptics to block PCP was strongly correlated with their ability to block tryptamine-induced seizures, a presumed measure of antiserotonergic activity. PCP-blocking activity was also correlated with inhibition

of spiroperidol binding in the frontal cortex and inhibition of LSD binding both in which reflect antiserotonergic activity. In addition, significant correlations were also obtained with several dopaminergic measures, particularly inhibition of dopamine binding. This correlation, however, cannot be considered to indicate that PCP interacts directly with dopamine binding sites. These findings, therefore, suggest that a combination of anti-serotonergic and antidopaminergic activity is important for blocking the effects of PCP. This does not necessarily suggest that PCP acts directly on dopaminergic and serotonergic systems.

Significance to Biomedical Research and the Program of the Institute

Our work in the drug abuse area has focused on the effects of alcohol consumption and the use of phencyclidine (PCP). The significance of performing research on these two substances becomes readily apparent when considering the pervasiveness of their misuse and the damage to the individual, family and society that their abuse brings.

Turning first to phencyclidine, we find that phencyclidine hydrochloride intoxication, especially among young people, has reached alarming and epidemic proportions. Abuse of PCP, often referred to as "angel dust" or "hog", frequently appears to result in violent behavior and mortality among its users. Consumption of PCP has a profound effect on mental status and is known to cause disorientation, psychosis, uncontrolled violent reactions and convulsions.

Because these violent reactions have been reported to last, in certain individuals, up to several weeks, pharmacological treatment is indicated. There is controversy, however, about a preferred form of treatment. Some clinicians recommend benzodiazepines, others recommend neuroleptics. Because of this treatment controversy, our findings relating to the differential effects of various neuroleptics on the amelioration of PCP-induced symptoms is of use to the medical community as well as the public, at large.

Our work with alcohol also is highly significant, particularly in light of the well known statistic that there are in excess of ten million alcohol abusers in this country. And, even though we have rather precise estimates on the extent of alcohol abuse, we have little definitive information on the effects of alcohol on memory. Thus, our work, beginning to differentiate those mental functions most severely hindered by alcohol consumption, is of importance to both the general population and the scientific community.

Proposed Course

We plan to continue investigating the effects of various pharmacologic treatments on the symptoms of phencyclidine-induced psychosis, as well as the differential effects of alcohol consumption.

PUBLICATIONS

Parker, E. S., Morihisa, J. M., Wyatt, R. J., Schwartz, B. L., Weingartner, H., and Stillman, R. C.: The alcohol facilitation effect on memory: A dose response study. Psychopharmacology 74: 88-92, 1981.

Hastroudi, S., Parker, E., DeLisi, L. and Wyatt, R. J.: On elaboration and alcohol. J. Verb. Lrng. and Verb. Behav. (in press).

Stoff, D.M. and Wyatt, R.J.: Interaction of monoamine blocking agents with behavioral effects of N,N-dimethyltryptamine. Psychopharmacol. Bull. (in press).



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01338-04 SMRA |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies of Aging | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R.J. Wyatt, Chief, Adult Psychiatry Branch, SMR; J.C. Gillin, Deputy Chief, APB, SMR; Staff Psychiatrists, APB, SMR, W.B. Mendelson, D. Shore, D.R. Weinberger, T.P. Bridge, D.V. Jeste and N.R. Cutler; Staff Fellows, APB, SMR, B. Phelps, A. Fine, A. Church, W.J. Freed and A. Sostek, G.N. Ko; Visiting Associate, APB, SMR, L. de Madinacelli. OTHER: J. Brodie, NIA; E. Parker, NIAAA; S. Rappoport, NIA; B.J. Hoffer, University of Colorado; J. Olson, Karolinska Institute; A. Seiger, Karolinska Institute; H. Weingartner, DCBR. | | |
| COOPERATING UNITS (if any) National Institute of Neurological Disease, National Institute of Aging, National Institute on Alcohol and Drug Abuse, Karolinska Institute, University of Colorado | | |
| LAB/BRANCH Adult Psychiatry Branch | | |
| SECTION Unit on Geriatric Psychiatry | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 12 | PROFESSIONAL: 8 | OTHER: 4 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The following report is an overview of the research studies initiated and the up-date of on-going studies by the Unit of Geriatric Psychiatry for the 1981-1982 reporting period. | | |

Objectives:

The objectives for research in the Unit of Geriatric Psychiatry are to test existing hypotheses and create new hypotheses relating to the social, psychological, cognitive and affective changes that occur through the aging process. Further, it is our objective to perform research that illuminates the differences between normal aging and pathology, synthesizing work from specific disciplines as well as interdisciplinary efforts.

I. Senile DementiaAluminum, Floride and Alzheimer's Disease

As we stated in last year's Annual Report, the most common cause of "senility" in the elderly is a degenerative brain disease called Alzheimer's disease or Alzheimer-type senile dementia. This illness is characterized by a progressive decline in memory and intellect, and a deterioration of social, occupational and communication skills. It is estimated that three to five percent of the United States population over age 65 is afflicted with this disease. Many more Americans will be afflicted as the average age of our population continues to rise. There is, presently, no effective treatment for Alzheimer's disease.

In recent years, patients with Alzheimer's disease have been found to have accumulations of aluminum in the hippocampus and cortex of the brain. These accumulations are localized within the nuclei of those nerve cells showing the neurofibrillary degenerative changes typical of Alzheimer's disease. This degeneration is most commonly seen in areas of the brain that are associated, generally, with memory and higher mental functions.

When certain aluminum salts are injected into susceptible animals, aluminum accumulates on DNA in the nucleus within nerve cells and neurofibrillary degeneration and learning impairments are seen. Aluminum will bind to DNA in a test tube, leading to destabilization of the normal DNA structure. This destabilization can be reversed by removing the aluminum, allowing the DNA to return to its normal configuration. Neurons with neurofibrillary degeneration (due to aluminum injections in animals or to Alzheimer's disease in man) are functionally impaired but do not rapidly deteriorate and die. This derangement of the many nerve cells with neurofibrillary degeneration may contribute to the severe mental impairments seen in patients with Alzheimer's disease.

In our work last year investigating the significance of the relationship between aluminum accumulations and Alzheimer's disease, we measured serum aluminum concentrations found in hospitalized patients of similar age and gender. The results tended to confirm that the increases in nerve cell nucleus aluminum reported in Alzheimer's disease are not the result of a generalized overload of this metal in biological fluids.

These findings, however, did not eliminate many of the questions concerning aluminum's role in the etiology and progression of Alzheimer's disease. If aluminum is a contributor to the degenerative brain changes in Alzheimer's disease, attempts to remove this metal from the body could have significant effects on the course of the illness. In this regard, the fluoride ion is of particular interest since elimination of aluminum by urine and feces is significantly increased by fluoride and aluminum retention in the body, reportedly, is decreased. A mutual reaction may occur between these ions in the body, resulting in the

formation of an aluminum fluoride complex with the result that aluminum is not retained in the organism.

To examine this possibility further we have initiated several preclinical studies investigating the aluminum-fluoride complexes. For several reasons, we have focused on the use of fluoride to complex the aluminum which has already been absorbed, rather than trying to prevent the absorption of aluminum. One factor was our interest in identifying patients early in the course of AD and attempting to prevent the further progression of dementia. Our earlier work showed that such patients do not have elevated concentrations of aluminum in blood or cerebrospinal fluid. Since most foods contain only 1.6 to 30 mg Al/kg, and normally only small amounts (less than 5%) of aluminum are absorbed, we have been more concerned with the potential toxicity of that aluminum already present in AD patients. Such patients may have a "vulnerability" to aluminum neurotoxicity on the basis of genetic, viral, or other inability to prevent aluminum from accumulating on DNA in neuronal nuclei.

In our first series of rabbits, aluminum was injected intrathecally, after which half of the rabbits were given p.o. F supplements (3 mg/kg/day in their drinking water), the other half received tap water. There were no differences in brain aluminum concentrations (in frontal, occipital, cerebellar, or hippocampal areas) determined by flameless atomic absorption spectrophotometry, between the two groups when they were sacrificed three weeks later.

In our series of rabbits, half were given 3 mg/kg of F in their water in nine days before all rabbits received intrathecal Al injections. At that time, the F pretreated rabbits had at least a 2-3 fold increase in CSF ionic F concentration over control rabbits. The control rabbits again received tap water, the F-pretreated group continued to receive supplemental p.o. F until all rabbits were sacrificed three weeks after the Al injections. Results from four brain areas (assayed on a blind basis) showed that F-pretreated rabbits tended to have lower Al concentrations, although the difference was statistically significant ($p=0.0083$) only in the cerebellum, with a non-significant trend favoring the F pretreated group in the hippocampus (see Table 1). Since the number of animals studied was so small and neuro-pathological changes were quite varied, we plan to repeat the study with a more adequate statistical design to discover whether p.o. F can prevent Al-induced neurotoxicity and again reduce Al accumulations in the brain.

Table 1

Brain Aluminum Concentrations
(Mean \pm SEM in $\mu\text{g/g}$ dry wt)
In Fluoride Pretreated and Control Rabbits

| | Cerebellum | Occipital Cortex | Frontal Cortex | Hippocampus |
|------------------------------|----------------|---------------------|-------------------|---------------|
| Fluoride Pretreated (N=5) | 8.8 \pm 2.1 | 11.6 \pm 4.7 | 5.8 \pm 2.4 | 4.1 \pm 2.0 |
| | * | | | T |
| Controls | 24.4 \pm 4.0 | 9.8 \pm 3.1 | 8.3 \pm 1.6 | 9.2 \pm 2.1 |

* $p=0.0083$, $t=3.65$

T $p=.1255$, $t=1.73$

We are also conducting a double-blind placebo-controlled study of the ability of fluoride to prevent the progression of early AD in outpatients. We are not yet able to conclude whether such treatment has a significant effect on the course of AD. We project that the results of our clinical study will be available in the next 1-2 years.

Pursuing the role of aluminum in Alzheimer's disease from another perspective, we became interested in examining levels of parathyroid hormone (PTH) in senile dementia patients. Much of the recent interest in aluminum toxicity has focused on an understanding of dialysis dementia. Dialysis dementia is a rapidly progressive and generally fatal disorder in which dysarthria, myoclonus and seizures are frequently observed. Aluminum in blood and brain reaches very high concentrations. Since parathyroid hormone is elevated in patients with renal failure and PTH has been correlated with serum aluminum concentrations in dialysis patients, the effects of this hormone on aluminum distribution have been studied. It has been shown that the absorption of aluminum from dietary sources is increased by PTH and that PTH causes aluminum to accumulate in kidney, muscle, bone and gray matter of the brain. It also has been suggested that PTH elevations might be responsible for increased tissue aluminum in senile dementia.

To investigate whether PTH is, in fact, elevated in SD patients, we measured the serum PTH concentrations along with renal and electrolyte indices. Ten patients between 66 and 82 years of age made up the SD group. These patients were diagnosed as having SD by ward psychiatrists and met DSM III criteria for this diagnosis. In addition, the Mental Status Questionnaire was administered to document organicity, and all members of the SD group had scores of 3 or less (out of a possible 10). Six inpatients between 65 and 85 years of age made up one control group. These patients lived on the same wards as SD patients, but had diagnoses of functional psychosis (mostly chronic schizophrenia). With the exception of two patients unwilling (or unable due to deafness and poor vision) to answer Mental Status Questionnaire items, all members of this group scored 7 to 10, suggesting little or no organic impairment. Four inpatients aged 65 to 71 made up another control group. They had dementias or lifelong mental retardation, but their organic impairment was clearly not due to SD. These patients lived on the same wards as the other two groups and were included in the study to determine whether differences in PTH (if found) between SD and chronic schizophrenia groups were related to dementia in general or to SD in particular. The schizophrenic and the non-Alzheimer dementia (or mental retardation) groups had generally been symptomatic longer and had longer periods of hospitalization. No patients with severe renal failure (creatinine > 2.2 mg% or BUN > 45 mg%) were included in the study. Since aluminum compounds may decrease serum PTH, we also excluded patients who had received aluminum-containing antacids during the past 3 months. All medications, taken by patients during the month before the study, were noted.

Venous blood samples were collected between 9 and 11 a.m. Large vacutainer tubes were immediately placed on ice and, after clotting, serum was separated in a refrigerated centrifuge and stored at -50°C. In order to determine if PTH values reflect differences in renal function or serum calcium (Ca) or phosphorus, blood for automated Technicon SMA II chemistry profiles was drawn simultaneously with PTH blood samples.

Serum immunoreactive PTH was measured using a biterminal assay. In order to assure reliability, duplicate measures were performed on each sample; there was less than 5% variability in the PTH values reported here. Serum concentrations in normal controls rarely exceed 120 μ l eq/ml, and only about 5% of normals have PTH concentrations greater than 100 μ l eq/ml. The reported levels of PTH mainly represent C-terminal immunoreactivity and do not necessarily reflect PTH activity.

All samples were analyzed on a blind basis within 5 weeks of collection. Time in storage did not correlate (Pearson $r=.02$) with PTH values.

We found that mean PTH values for each of the elderly groups were considered "high-normal" compared with healthy young controls. This increase probably is due (at least in part) to differences in renal function, since the mean PTH values for those elderly patients with creatinines below 1.5 mg% fell much closer to the PTH concentrations of young control subjects.

The control group with non-Alzheimer dementia (or lifelong mental retardation) did not differ significantly (two-tailed, t-test) from controls with functional psychosis with respect to age, serum albumin, or PTH. For this reason, data from both of these control groups were combined for comparison with the SD patients. Data were analyzed for 10 patients with SD of the Alzheimer type and 10 controls (4 with dementia or mental retardation and 6 with functional psychoses). The SD and control groups did not differ significantly on a test for homogeneity of variance or t-tests on the following variables: age, creatinine, BUN, inorganic phosphate (PO_4), total Ca, Ca-inorganic PO_4 ratio, PTH, or albumin.

Pearson correlation coefficients were used to determine the relationships between BUN, serum creatinine, Ca and inorganic PO_4 , and PTH values for the SD patients and the controls. Within the SD group, PTH correlated ($r=.73$, $p<.02$) with serum creatinine. This relationship did not hold for the controls ($p=.06$, $p<.85$). On the other hand, there was a significant inverse relationship between inorganic PO_4 and PTH values in the controls ($r=-.66$, $p<.04$) but not in the SD patients ($r=-.21$, $p=.57$). The Ca-inorganic PO_4 ratio was also significantly correlated ($r=.79$, $p<.01$) with PTH in the control group but not in the SD patient group ($r=.14$). Neither BUN nor total Ca correlated significantly with PTH values for either group.

The correlation between creatinine and serum PTH is not surprising but it is curious that this relationship was significant in the SD patients but not the controls. When we recalculated the correlation between creatinine and PTH excluding SD patients with creatinine 1.5 mg%, the creatinine-PTH correlation disappeared ($r=.08$). Similarly, since PTH promotes the excretion of phosphate and causes an increase in serum Ca, the inverse correlations of inorganic PO_4 with PTH and the positive correlation of the total Ca-inorganic PO_4 ratio with PTH in controls were not unexpected. Neither of these relationships, however, was statistically significant in the SD patients. Again, eliminating the SD patients with creatinine > 1.5 mg% increases both the inverse correlation between PTH and inorganic PO_4 ($r=-.48$) and the positive correlation between PTH and Ca/inorganic PO_4 ($r=.42$), though neither probability was less than .10 in these SD patients.

Since the SD group had a non-significant trend towards being older than controls, we calculated age-PTH correlations for SD patients and controls. Neither of these correlations approached significance. There have been reports, though, of PTH increases with age from the 3rd to 8th decade and that the increase in PTH above age 70 is more pronounced in women than men. While our data do not show this effect, the narrow age range (65 to 85 years) and small N (10) of our patient group would make statistically significant correlations difficult to demonstrate.

Another finding of interest is that while most SD patients have PTH values in the same range as age matched controls, two SD patients have considerably higher PTH immunoreactivity. While both of these high-PTH SD patients have elevated creatinine and BUN, these

renal indices alone cannot account for the PTH values. It is clear that the two outlying SD patients still have elevated PTH values even when compared with other patients having similarly high BUN and creatinine values. The high PTH values in these two patients are not suspected to be secondary to medications as the only drug that both patients were taking was a hospital formulary laxative preparation which was also being taken by several other patients with low PTH.

These findings suggest the possibility that patients diagnosed as Alzheimer type SD - even by rigorous psychological criteria - may belong to a heterogeneous group. It may be that the two patients with relatively high PTH values might have high aluminum concentrations in cerebral cortex without large numbers of senile plaques or tangles. This pattern of high serum PTH and CNS aluminum with few plaques and tangles is typical of dialysis dementia and could be indicative of similar pathophysiology.

Alternatively, the PTH elevations may be fortuitous. Both of the SD patients with elevated PTH values had low serum albumin concentrations (3.0 and 3.5 gm%) in addition to raised serum creatinines. Since none of the control patients with elevated creatinines had such low serum albumin levels, we investigated the effects of varying albumin concentrations on the PTH assay. Six different concentrations of human serum albumin were added to several serum samples and the assay was repeated. Variations in albumin concentration did not affect either specific or non-specific binding in this immunoreactive PTH assay.

We suspect that the low serum albumin in conjunction with high creatinine and BUN could reflect greater renal impairment in the two high-PTH SD patients. Additional evidence supporting this interpretation is the finding that both high-PTH SD patients had 3 to 4 (+) proteinuria, while other patients with elevated creatinine (but normal PTH) did not have proteinuria at the time bloods were drawn for PTH and chemistry profiles.

It cannot be assumed that the reported immunoreactive PTH values represent either the functional level of the parathyroid gland or the physiologic activity of circulating PTH. The assay measures mainly C-terminal immunoreactivity, which could be increased by several mechanisms. Increased release of active PTH from the parathyroid glands is one possible mechanism, others include increased generation of C-terminal reactivity due to altered PTH metabolism or deficient renal clearance of PTH or its fragments.

The possibility was raised that, rather than having very high serum PTH values, SD patients may have slight PTH elevations acting over many years to produce aluminum accumulation in the gray matter of the cerebral cortex. An accurate evaluation of this hypothesis requires testing in much larger sample sizes. In the present study, however, the only SD patients with increased PTH values had proteinuria and serum creatinine concentrations above the upper limit of the normal reference range. Also, subcellular aluminum localization in SD patients differ from that of dialysis patients. Aluminum in SD patients' brains is almost exclusively restricted to the nuclear chromatin fraction while dialysis dementia patients (with greatly elevated serum PTH) have aluminum accumulation mainly in the cytoplasm.

In conclusion, while elevated circulating PTH may lead to aluminum accumulation in the brain, it does not seem to be a factor in the etiology of SD (Alzheimer's disease). The slight (non-significant) differences in PTH between SD and age and gender matched control groups appear to be due to greater renal impairment in two of our SD patients.

In another related study, we again examined the relationship between serum aluminum (Al) and primary degenerative dementia (PDD) while controlling for age, gender and hospitalization. Charts were selected and reviewed when a patient was diagnosed clinically as having PDD (either senile dementia or Alzheimer's disease). Records were examined to confirm the diagnosis and assure that patients were not exposed to any aluminum-containing antacids. Additional exclusion criteria included evidence of severe renal failure, marked anemia, or any other possible etiology for the dementia (e.g., vascular, endocrine, toxic-metabolic, etc.). The Mental Status Questionnaire (MSQ) version was given as a simple objective indicator of degree of organic impairment, and scores (0 to 10 for most to least demented) were recorded (see Table 1).

Table 1

Description of Patients and Controls with Serum Aluminum Concentrations

| Diagnosis | Age (years) | Gender | Race | MSQ Score | Mean Serum Al (ug/liter or ppb) |
|-------------------------------|-------------|--------|------|-----------|---------------------------------|
| Primary Degenerative Dementia | 80 | F | B | 0 | 26.6 |
| | 78 | F | W | 0 | 9.6 |
| | 66 | F | B | 0 | 5.8 |
| | 78 | F | P | 0 | 9.3 |
| | 73 | F | W | 3 | 5.6 |
| | 92 | F | B | 0 | 8.1 |
| | 79 | F | P | 0 | 5.7 |
| | 70 | F | B | 0 | 11.85 |
| | 75 | F | P | 0 | 3.0 |
| | 79 | F | B | 0 | 4.45 |
| | 69 | F | P | 0 | 3.9 |
| | 84 | M | W | 0 | 2.25 |
| | 82 | F | B | 0 | 3.9 |
| | 84 | F | W | 0 | 3.9 |
| (early) | 71 | F | P | 5 | 2.75 |
| Group 1 | Mean 77.3 | | | Mean 0.53 | Mean + SD = 7.11 + 5.89 |
| Mental Retardation | 71 | F | W | 4 | 6.5 |
| | 65 | F | W | 0 | 5.05 |
| Epilepsy, A-V Malformation | 69 | M | B | 3 | 5.8 |
| Meningioma | 68 | F | P | 0 | 2.2 |
| Korsakoff's Syndrome | 71 | F | B | 0 | 7.5 |
| Group 2 | Mean 68.8 | | | Mean 1.3 | Mean + SD = 5.41 + 7.80 |

| | | | | | |
|---------------------------------|-----------|---|---|----------|----------------------------|
| Depression | 71 | M | B | 8 | 6.8 |
| Chronic Schizophrenia | 69 | F | B | 7 | 11.1 |
| | 73 | F | W | 10 | 5.0 |
| | 74 | F | B | refused | 5.65 |
| | 70 | F | W | refused | 2.65 |
| | 64 | F | W | 10 | 6.7 |
| Manic-Depressive, Manic Type | 64 | F | B | 7 | 5.65 |
| Functional Psychosis | 85 | F | B | 7 | 3.9 |
| Group 3 | Mean 71.2 | | | Mean 8.3 | Mean + SD = 5.93 + 2.34 |

These individuals ranged in age from 64 to 85 years (mean = 71, n=8), and those willing to answer MSQ items scored between 7 and 10.

One-way analysis of variance showed the three groups to differ in age ($F=4.56$, $p=0.02$). The non-PDD demented patients (Group 2) were significantly younger (two-tail t-test, $p < 0.05$) than the PDD patients (Group 1). However, there were no significant differences in age between the functional psychosis patients (Group 3) and either of the other two groups.

Pearson correlation coefficient ($r=0.91$, $p < 0.0001$) was calculated for serum Al blind duplicates from 22 of the 28 subjects (for 6 subjects only 1 value could be reported) and indicated good reliability for this assay. The means from duplicates ranged from 2.2 to 26.6 ug Al/liter (parts per billion or ppb). Table 1 lists each subject's age, race, gender, and serum Al values. An analysis of variance revealed no significant difference in serum Al concentrations between the three groups. Also, consistent with our preliminary data, we found no significant age-serum Al correlations. One senile dementia patient had a relatively high serum Al. In general, however, we found that elderly demented (Group 2) and non-demented (Group 3) inpatients all had serum Al concentrations in the same general range as did the PDD patients.

With the exception of one patient, we find no difference between serum Al concentrations in PDD patients and age-matched demented or non-demented controls. This "outlier" had the greatest degree of renal impairment of any patient in our study, with elevated serum creatinine and BUN, and low serum albumin with 3-4 (+) proteinuria. Since Al excretion is reported to occur via the kidneys, decreased Al elimination is one explanation which might account for our outlier's elevated serum Al.

Our failure to find significant differences in serum Al in senile dementia patients is consistent with the work of other researchers in this area. We find no indication that the reported elevations of cerebral cortex Al in PDD patients are the result of a generalized tissue overload as may occur in patients on dialysis. While dialysis dementia patients have extremely high serum Al concentrations they do not develop the senile plaques and neurofibrillary tangles typical of PDD. It has been reported that CNS Al accumulation in Alzheimer's disease (PDD) is almost exclusively localized to the nuclear chromatin (DNA) fraction, while in dialysis dementia the Al is predominantly cytoplasmic. It has been reported also that neurons with neurofibrillary tangles have high Al concentrations in their nuclei. Aluminum attachment to DNA in the brain might be due to preexisting abnormalities of the

chromatin. Another possibility is that a failure of the blood-brain barrier may allow for Al accumulations.

In conclusion, while abnormal accumulation of Al in PDD (senile dementia or Alzheimer's disease) patients' brains may occur, the reasons for and significance of this accumulation remain unclear. Our findings of normal serum and CSF Al in PDD patients indicates that it is unlikely that nuclear Al accumulation is the result of a generalized overload of this metal in biological fluids. Further study of the interaction between Al and CNS nuclear chromatin may show why the Al attaches itself, whether this attachment precedes or follows neurologic deterioration, and how cortical Al accumulation might be prevented or reversed.

II. Neuroleptics

Tardive Dyskinesia

Tardive dyskinesia (TD) is considered to be a major complication of long term neuroleptic treatment. Although the introduction of neuroleptics in the mid-1950's is believed to have played a major role in the dramatic decline in the numbers of hospitalized schizophrenics, the overall prevalence of tardive dyskinesia has increased since that time to its present and serious level. It is more likely to occur in the elderly on neuroleptic treatment.

Our work in the area of tardive dyskinesia continues to investigate:

- (1) Clinical differences, especially concerning epidemiological variables, history of psychiatric illness and its treatment, physical status, blood-neuroleptic level and platelet monoamine oxidase activities, organic brain damage as judged by psychological testing and CAT (computerized axial tomography) scan between dyskinesia and non-dyskinesia patients.
- (2) If criteria can be determined to categorize patients into subgroups of risk/no risk patients.
- (3) Potential side effects of specific neuroleptic drugs (e.g., thioridazine) and
- (4) The relationship, if any, of neuroleptic treatment and retinitis pigmentosa.

In previous studies we have determined that:

- (1) Tardive dyskinesia is significantly correlated with high blood serum levels of neuroleptic drugs,
- (2) There appears to be no significant correlation between tardive dyskinesia and obvious structural brain abnormalities, and
- (3) The toxic side effect of retinitis pigmentosa has not been found to be associated with the administration of thioridazine.

We have initiated new research to investigate possible correlates with TD that might provide possible trait, not state, markers for predisposition. Fifty elderly psychiatric females were screened, identifying eight with severe TD. Eight matched controls were found. Bio-

chemical and CAT scans were performed and these subjects have been projectively followed for one year.

There were no significant differences found between groups on CAT scan readings. There were significantly higher levels of serum DBH with thioridazine in the TD group while MAO levels in this group were lower. There were no significant drug effects for other drugs. Also, within individuals, other markers were stable over time.

In a prospective study, subjects without symptoms of tardive dyskinesia were screened and selected. Of this group, the one individual with the highest DBH level at the outset of the study has since developed TD. This raises the question of whether or not DBH could be a marker for a predisposition to tardive dyskinesia.

In research using a liquid chromatographic assay, we measured serum neuroleptic concentrations in eight middle-aged and elderly female inpatients with tardive dyskinesia (TD), and eight controls. All 16 patients were receiving either thioridazine and mesoridazine at stable doses. TD patients were found to have a significantly higher ratio of serum concentration to daily dose of neuroleptics, compared with controls. A one-year follow-up revealed that this ratio did not change appreciably in those patients who continued to receive neuroleptics. Differences in serum neuroleptic levels were not related to peripheral inflammatory activity as indicated by serum α -1-acid glycoprotein concentrations. Of the various thioridazine-metabolites, sulforidazine which is reportedly the most potent one in terms of affinity for dopaminergic and α -noradrenergic receptors, seemed to be significantly elevated in the serum of TD patients as compared with non-TD patients. Our data suggest a need for further pharmacokinetic investigations to study neuroleptic metabolism in patients with TD.

Looking to a somewhat different direction, using the Bender-Gestalt test as the dependent measure, we hypothesized that a group of chronic schizophrenic patients with tardive dyskinesia would show more perceptual dysfunction than those without. To test this hypothesis, we selected twenty (12 male and 8 female) chronic schizophrenic patients. These patients ranged in age from 21 to 72 years with a mean age of 39.4 years. The Research Diagnostic Criteria for chronic schizophrenia were satisfied. Without previous knowledge of tardive dyskinesia status, all 20 patients were assessed for perceptual functioning on the Bender-Gestalt test. Gross distortions of the Bender reproductions were categorized as "organic," whereas those with no distortions were categorized as "non-organic." These two categories, however, were not mutually exclusive. Degree of distortion, e.g., low vs moderate, determined placement in the nonorganic vs organic categories, respectively.

Since the Bender measures perceptual-motor behavior and since irregular hand movements are characteristic of tardive dyskinesia, it was essential to control for the effects of motor irregularities on the Bender. Consequently, we used Hutt and Briskin's discriminators of perceptual disturbance, i.e., rotation, fragmentation, perseveration, lack of closure, and angulation difficulties, not irregularities of line, as selection criteria for the organic category.

Nine of 10 patients with tardive dyskinesia and 6 of 10 patients without tardive were correctly identified on the basis of their organic and nonorganic protocols, respectively ($\chi^2=5.49$, $p<.02$). There were no significant differences between groups with and without symptoms across the variables of age, gender or length of neuroleptic treatment. Four times as many false inclusions in the group without compared with the group with symptoms

suggests that the Bender is more reliable in identifying the presence of tardive dyskinesia than its absence. The less accurate identification of organic and nonorganic discriminators in the non-tardive dyskinesia group is consistent with the finding that within any chronic schizophrenic group there is a subgroup of patients with brain damage, an approach which would also explain the overlap between organic and "schizophrenic" discriminators on the Bender. In terms of predisposition to tardive dyskinesia, we are not suggesting CNS dysfunction accounts for the total variance, since predisposition to any disorder is a multivariate phenomenon, but CNS dysfunction could be one variable in predisposing some individuals to tardive dyskinesia. CNS dysfunction could also be secondary to the clinical state of tardive dyskinesia. If future research indicated that withdrawal of neuroleptics reversed the symptoms of tardive dyskinesia but not the visual-motor dysfunction on the Bender, it could be argued that the dysfunction is not related to the clinical state of tardive dyskinesia.

These preliminary findings suggest that, although the Bender is a reliable measure of tardive dyskinesia according to the present binary, i.e., organic vs nonorganic, classification, there is a clear need for greater objectivity in the quantification of perceptual, pathognomic distortions, along with appropriate cutting scores to discriminate tardive dyskinesia from non-tardive dyskinesia patients. This work is currently being undertaken.

Although the prevalence of TD among neuroleptic-treated inpatients in the western countries has been about 26%, little is known about its pathophysiology. One of the conditions that is thought to be a human model for neuroleptic-induced TD is levodopa-induced dyskinesia (LID) in patients with Parkinson's disease. There is, however, a scarcity of studies comparing clinical characteristics of these two iatrogenic dyskinesias. We, therefore, performed the following investigation of similarities and differences between TD and LID.

Thirty Parkinson's disease patients with LID (17 males, 13 females, mean age (+ SD) 57 + 13 years) were seen as outpatients. All were maintained on their normal regimen of levodopa and carbidopa (Sinemet). In addition, many patients received other parkinsonian therapy such as amantadine, anticholinergic and tricyclic antidepressant medications. Twenty-four patients received trials of ergot derivatives with dopamine agonist properties (bromocriptine, lisuride, or pergolide) adjusted to the optimal antiparkinsonian dose. In all cases the dyskinesia first occurred while the patient was receiving levodopa or Sinemet and was often the limiting factor in Sinemet treatment.

Twenty-five psychiatric inpatients (20 chronic schizophrenic and five organic brain syndrome and tardive dyskinesia patients) were studied (nine males, 16 females, mean age 57 + 17 years). The length of their illness was computed from the patients' records using the time since first psychiatric hospitalization was necessary. Eight patients were medication free at the time of the study and the others were receiving neuroleptic medications. All had received long term neuroleptic treatment, the length of which was determined by chart review. Because of difficulties in separating schizophrenic symptoms from those associated with chronic institutionalization and aging, we did not attempt to grade the severity of schizophrenia.

The severity of TD was determined using the Abnormal Involuntary Movement Scale (AIMS). The AIMS groups the musculature into seven regions (muscles of facial expression, lips, jaw, tongue, upper extremity, lower extremities and trunk). Ratings range from 0 (absent) to 4 (severe) for each area (total possible score = $7 \times 4 = 28$). Separate raters scored the patients with LID and TD, simultaneously evaluating 21 episodes of dyskinesia (11 pa-

tients with LID, 10 with TD). The intraclass correlation coefficient for the two raters was .84 for total score.

Table 1 compares the demographic variables in the two dyskinesia groups. The mean total AIMS score did not differ significantly between patients with LID (9.3 ± 5.0) and those with TD (11.6 ± 4.0 , $t=1.80$, $p < .07$). However, the sum of facial area scores (muscle of facial expression, lips, jaws, and tongue) of patients with TD (8.0 ± 2.6) was significantly higher than that of patients with LID (4.4 ± 2.7 , $t=5.0$, $p < .0001$). The sum of the scores for extremities and trunk (limb-truncal score), for the patients with LID (4.8 ± 2.8) tended not to be significantly elevated compared to scores of patients with TD (3.6 ± 2.6). Table 2 compares the presence or absence of dyskinesia in each anatomical area. Patients with TD had an increased proportion of patients with lip and tongue movements and a decreased proportion of patients with movements in the lower extremities.

We also examined the relationship between parameters such as age, length of illness, length of medication treatment and severity of dyskinesia. Each of these parameters was correlated (Pearson's r) to the separate dyskinesia scores for both patient groups. The only significant correlations observed were between length of Parkinson's disease and dyskinesia scores for the limb-truncal area ($r=.61$, $p < .001$) and for the entire body ($r=.54$, $p < .01$).

The final relationship examined was that between degree of parkinsonian disability and dyskinesia scores in parkinsonian patients with LID. Because of the significant correlation between length of parkinsonism and dyskinesia score a one-way analysis of covariance was performed using the length of illness as the covariate. There was a significant effect of stage of disability on the total dyskinesia score ($F=4.15$, $p < .03$) and limb-truncal score ($F=3.94$, $p < .03$) but not the facial score ($F=2.40$, $p > .10$). The adjusted means are given in Table 3. As can be seen from the table stage I patients have dyskinesias which are significantly less severe than patients with more severe parkinsonism.

Investigating, further, the relationship of tardive dyskinesia to pharmacological agents, we studied acute behavioral and biochemical effects of two dopamine agonists, bromocriptine and apomorphine, administered in low doses to hospitalized chronic schizophrenic patients with and without movement disorders. Of the 12 patients studied, four had neuroleptic-induced tardive dyskinesia and one had neuroleptic-induced Gilles de la Tourette's syndrome. We sought answers to two questions: (1) Do low doses of bromocriptine and apomorphine significantly reduce neuroleptic-induced abnormal movements? (2) Do patients with movement disorders respond differently from those without movement disorders, in terms of psychosis ratings and biochemical parameters?

We gave bromocriptine (2 mg orally), apomorphine (0.01 mg/kg and 0.005 mg/kg subcutaneously) and placebos (one each for bromocriptine and apomorphine) on different days. The order of drug administration was randomized, and assessments were gathered under double-blind conditions. Two independent raters repeatedly assessed the symptoms on the Abnormal Involuntary Movements Scale (AIMS) and the Brief Psychiatric Rating Scale (BPRS) throughout the evaluation period beginning 1/2 hour before until two hours after the treatment. Blood was also collected at regular intervals for biochemical measures (primarily catecholamine metabolites in plasma).

Preliminary analysis of data shows significant reduction in both AIMS and BPRS ratings with bromocriptine in the patient with neuroleptic-induced Tourette's syndrome. The patient had no significant response either to apomorphine or to placebo. Other behavioral and biochemical data are being analyzed.

Table 1
Comparison of the Two Dyskinesia Groups

| <u>Patients with LID</u> | Length (years) |
|--------------------------------|-------------------|
| Age | 57 \pm 13 |
| Length of Illness | 13 \pm 5 |
| Length of Medication Treatment | 9 \pm 3 |
| <u>Patients with TD</u> | |
| Age | 57 \pm 17 |
| Length of Illness | 28 \pm 15 |
| Length of Medication Treatment | 14 \pm 7 |

Values represent mean \pm SD

LID=Levodopa-induced Dyskinesia

TD=Tardive Dyskinesia

Table 2
Frequency of Involvement of Different Regions in LID and TD

| | Muscle of Facial Express | Lips | Jaws | Tongue | Upper Ext. | Lower Ext. | Trunk |
|-----------------------------------|-----------------------------|------|------|--------|---------------|---------------|-------|
| Patients with LID (total n=30) | 13 | 13** | 13 | 4** | 18 | 23** | 14* |
| Patients with TD | 11 | 22 | 15 | 20 | 14 | 6 | 6 |

*p < .1

**p < .001 Fisher exact probability test

LID=levodopa-induced dyskinesia

TD=neuroleptic-induced tardive dyskinesia

Values represent of patients who had a mean rating of at least 2 for a particular region on the 0-4 AIMS.

Table 3

The Severity of Parkinsonian Symptoms and Dyskinesia in
Parkinson's Disease Patients with Levodopa-Induced Dyskinesia

| Stage of Symptoms | I (n=11) | II (n=12) | III and IV (n=7) |
|-----------------------------|------------------|--------------|---------------------|
| Mean Total Score | 6.2 ¹ | 11.2* | 10.9* |
| Mean Facial Movements Score | 3.0 | 5.5 | 4.7 |
| Mean Limb-Truncal Score | 3.2 | 5.6* | 6.0** |

¹Means are adjusted for length of illness. No standard deviation is given.

* $p < .05$ Scheffé test compared to stage 1 patients.

** $p < .01$

III. Parkinson's Disease

A. Brain Grafts

Our work on tissue brain grafts has continued to expand since last year's Annual Report. As has been explained previously, rats with unilateral lesions of substantia nigra pars compacta (SN), the area of the brain containing most dopamine-containing neurones, are a widely recognized animal model of Parkinson's disease. When given dopamine agonists such as apomorphine, these rats rotate in a direction contralateral to the lesion, presumably because of the development of supersensitive dopamine receptors in the striatum ipsilateral to the lesion. When grafts of embryonic SN are placed in the lateral ventricle, or into a transplant cavity adjacent to the striatum in animals with SN lesions, this rotational behaviour has been shown to decrease. Histochemical examinations have shown that axons from the grafts have grown into the striatum, and biochemical measurements indicate that dopamine concentrations are increased in areas of the striatum adjacent to the SN grafts.

In our original work, cited in previous reports, we had transplanted fetal rat tissue into the denervated substantia nigra of adult rats. We have been watching the progress of these rats to assess the prolonged survival and success of the transplants.

The rats were assessed behaviorally at six months and histologically at 9 months. Behaviorally, the animals continued to show significantly decreased rotation. Histologically, the grafts were studied for dopamine content and were found to be producing large amounts and reinnervating the host caudate nucleus. Interestingly, at 8 to 9 months, the transplants, unlike the rest of the animal's brains, showed no signs of aging.

One obvious problem with this technique, however, both for basic research and possible clinical applications, is the requirement for fetal central nervous donor tissue. To circumvent this problem, we sought other cells to substitute for the fetal tissue. We found that the

adrenal medulla contained some cells with similarities to some substantia nigra cells. There are several reasons for considering the adrenal medulla as a potential replacement for fetal SN grafts. First, the normal adrenal medulla produces dopamine as an intermediary in the synthesis of adrenaline. Second, adrenal chromaffin cells, which are normally rounded in shape, become angular and develop processes when grown as grafts in the anterior eye chamber or when grown in culture in the absence of corticosteroids. Finally, processes originating from intraocular adrenal medulla grafts can innervate intraocular grafts of cerebral cortex.

We produced unilateral substantia nigra lesions in male Sprague-Dawley rats by stereotaxic injections of 6-hydroxydopamine hydrobromide into the right SN. Approximately 2 months later, the animals were tested for apomorphine-induced (0.1 and 0.25 mg per kg subcutaneously) rotational behaviour using an automated apparatus. The smaller of the two doses that produced at least 80 counterclockwise rotations in 40 min was used for each animal. Animals turning at less than this rate were not used. After the initial screening, rotational behaviour was re-examined for five to seven additional sessions to obtain a stable baseline for each rat.

Adrenal glands were removed from young adult Sprague-Dawley rats (125-200 g) and the medulla dissected free. A cut was made in the surface of the medulla to enable it to be opened, and the interior was removed. This method was used to avoid inclusion of the adrenal cortex in the graft. Four to six grafts were implanted in the lateral ventricle on the 6-hydroxydopamine-lesioned side in each of 13 rats (age at least 5 months). Control (n=15) animals received grafts of sciatic nerve instead of adrenal medulla.

Adrenal medulla (n=13) or sciatic nerve (control) grafts (n=15) were implanted and 2 months allowed for survival and growth of the graft tissue. At this time, rotational behaviour was examined for five 40-min sessions, and some of the animals killed for histochemical studies.

When the rats were tested 2 months after transplantation, rotational behaviour was significantly less in the animals with adrenal medulla grafts than in those with sciatic nerve grafts. Histochemical fluorescence studies of six randomly selected rats showed the denervations of the caudate nucleus to be essentially complete, with only a few fine terminals remaining in one rat. Grafts with catecholamine-containing chromaffin cells were found in five of the six animals. These cells had developed polygonal shapes and were usually grouped in one or more small clusters within the grafts. Two types of fluorescent cells were found in the adrenal grafts, similar to the noradrenaline (very strongly fluorescent) and adrenaline (moderately fluorescent) cells present in normal adrenal medulla. Some of these cells had fine elongated processes, but very few fibres were seen to leave the grafts and enter the host brains. The total numbers of chromaffin cells found in the grafts, were 880, 4,080, 66, 517, and 2,134, respectively, for the five animals. Adrenal cortex was also found in the graft that contained 4,080 chromaffin cells. Rotational behaviour was not reduced in this animal, nor in the animal that had only 66 chromaffin cells.

The observations suggest that adrenal medulla grafts can reduce lesion-induced rotational behaviour, even though there was no clear evidence that the grafts actually reinnervated the host caudate nucleus. Although the transplanted chromaffin cells were elongated and produced fine fibres, these fibres were found almost entirely within the grafts, and rarely penetrated into the caudate nucleus. This suggests that catecholamines or other substances diffused from the grafts to receptor sites in the caudate nucleus in sufficient

quantity to reduce caudate dopaminergic supersensitivity and consequently, apomorphine rotation.

To further substantiate the results of our grafting research, we have been seeking more sophisticated means of histologic examination. It is a relatively simple matter to locate and identify several tissue fragments transplanted within the central nervous system. Intraventricular grafts stand out as tissue islands within a fluid space, and even intraparenchymal grafts generally display sharp borders and histological appearance distinct from the surrounding host brain.

We, and workers in several other laboratories, have been interested in transplanting disassociated cells into brain and spinal cord. The task of identification, however, is difficult. If the cells have successfully integrated themselves within the host tissue, they will be morphologically indistinguishable. Some marker is necessary. Perhaps for this reason, the few reported experiments have used catecholamine-producing donor cells whose survival and development in host brain can be revealed by formaldehyde- or glyoxylic acid-induced fluorescence histochemical techniques. Fluorescence artifacts, however, abound. The characteristic emission of spectra of the catecholamine condensation products are valuable guides to correct attribution of fluorescence. Observed colors depend upon choices of filters, which ought to be specified with other suchose-phosphate glyoxylic acid method. For example, catecholamine fluorescence excited at 405 nm appears aquamarine when viewed through a 435 nm barrier, but apple green through a 495 nm barrier. In the latter case, the autofluorescence of macrophages may be mistaken for catecholamine fluorescence. That macrophages frequently migrate to the site of graft cell injection and may extend pseudopodia resembling neuronal arborization only make this artifact more hazardous to the unwary. Without color photographs, some published accounts of transplanted cell survival must remain unconvincing.

Because the color of non-specific tissue autofluorescence may be close to that of the catecholamines tissue debris or, irregularities in the section may be misleading, condensed cytoplasm, as in dying cell (even dying transplanted cells), may generate disturbing non-specific fluorescence. Such structures may appear as long axon-like processes, and be quite misleading. Without further tests of fluorescence specificity, photographs (especially black and white) identifying densely fluorescent "cells" as catecholaminergic are also unconvincing.

We have taken advantage of the patient-dependent shift in catecholamine fluorescence excitation to devise a simple test for specificity. Following glyoxylic acid treatment at neutral pH, dopamine and norepinephrine form quinoidal fluorophases whose broad excitation spectrum display maximum at 410 nm. The non-quinoidal fluorophases produced at acid pH have excitation maximum at shorter wavelength, 370 nm. At 450 nm, only the neutral form fluoresces. Thus a catecholamine containing structure will fluoresce using sharp excitation after at 405 and emission barrier at 435 nm, after processing at neutral or acidic pH. When excited at 430-450 nm and viewed through a 480 nm barrier, such structure fluorescence in neutral pH sections but not in acidic ones. Non-specific fluorescence persists at both pH ranges through both filter combinations. The abolition of long-wavelength-excited fluorescence by acidic pH, then, provides a stringent test for catecholamine-derived glyoxylic acid histofluorescence. Other tests have been proposed, including selective dissolution of catecholamine fluorescence by water, or its reduction by sodium carbohydrate. The documentation of such a specificity test is essential in any claim of identification of dispersed catecholamine-containing neuronal transplant. In the past, such demonstrations have been lacking.

By these stringent criteria, we have in fact successfully transplanted dispersed catecholamine containing cells within rat brains. Both embryonic substantia nigra, and neutral adrenal medulla, have been disassociated into single cell suspensions by enzyme incubation. Small volumes of such cell suspensions, typically 3-6 μ l, are injected smoothly over three minutes to sites within the host adult rat caudate, whose endogenous dopamine input has in some cases been previously destroyed. Two to four weeks later, surviving cells can be seen which pass the "acid test" for catecholamine fluorescence. Cells resembling characteristic adrenal chromaffin cells can be seen more clearly. In the other cells resembling normal substantia nigra dopaminergic neurons can be seen to elaborate lengthy processes within the host caudate.

B. Blinking

In related work, we explored the relationship between blink rates and parkinsonism, including the extent of disability, and the presence or absence of levodopa-induced dyskinetic symptoms among treated parkinsonian patients. The most severely bradykinetic parkinsonian subjects, in whom nigrostriatal dopamine is likely to be greatly depleted, might be expected to demonstrate the slowest rates of spontaneous blinking. Furthermore, if dyskinesia induced by levodopa is derived from post-synaptic supersensitivity to dopamine, then a similar phenomenon may be found with respect to blink rate as a manifestation of hyperkinesia. Hence, depression of blink rate should correlate with the degree of parkinsonian disability, and patients having levodopa-induced dyskinesia should have an increased spontaneous blink rate. To test this hypothesis, blink rates were examined in a group of parkinsonian subjects with varying degrees of disability.

Fifty-four parkinsonian subjects (35 males, 19 females) were seen as out-patients. The mean age (\pm SD) was 56 ± 13 years, and parkinsonism was symptomatic for a mean of 12 ± 9 years. Fifty-two of the group had been on treatment with levodopa and carbidopa for several years sometimes in combination with other drugs (including anticholinergics, tricyclic antidepressants and amantadine). Twenty-six of these patients received additional therapy with a D_2 agonist (either bromocriptine and lisuride). The regimen of dopamine agonists had been unchanged for two weeks or longer prior to assessment.

Twenty-four controls (14 males, 10 females; mean age 56 ± 12 years) were chosen from clinical staff, patients' family members (spouses and siblings) and three patients with other non-parkinsonian neurological disorders.

Blinks were counted and timed by an experienced observer for the first five minutes of conversation during routine examinations with a familiar interviewer: these were calculated as the mean number of blinks per minute. Although subjects sometimes were informed that "certain movements" were under observation, blinking was not specified.

Each patient was rated with a five point scale as to the overall severity of parkinsonian disability. The ratings were as follows: I) minimal or no functional impairment, tremor at rest may be a prominent symptom, and parkinsonism may be restricted to one side; II) mild degree of disability, with bilateral or midline involvement but no impairment of balance; III) moderate functional disability from parkinsonism; righting reflexes are impaired. Patient may be restricted in activities requiring walking, dexterity, and rapid movements; IV) fully developed, severely disabling disease; the patient may walk and stand unassisted in a precarious state; V) severe disability, with inability to walk. Other disabilities such as impaired speech, self-feeding, rigid state, and so on, may be present. Patients with stages III and IV disease were grouped together because of the small number of available patients in

each category, and there were no stage V patients in this study. The blink observer was not involved in the clinical staging of each patient.

Each patient was carefully observed for dyskinetic movements. If present, these were scored by a modified version of the abnormal involuntary movement scale (AIMS), omitting the use of the blinking index rating as one of the criteria for dyskinesia. With a rating of "2+" or more in any of seven major motor groups (muscles of facial expression, lips, jaw, tongue, arm, leg and trunk) a patient was designated as dyskinetic. There were no significant differences between age or mean duration of illness among patients in various stages of parkinsonism, or between the dyskinetic versus the non-dyskinetic groups.

The mean \pm SD for normal controls was 16 ± 9 blinks/minute. The stage of illness had a significant effect ($F(3,74)=3.78$, $p < .02$, one-way analysis of variance (ANOVA) for independent measures). The means, compared in a post-hoc fashion by the Scheffe test, demonstrated that patients with advanced parkinsonism had significantly decreased blinking as compared with normals ($t=2.37$, $p < .03$), or with stage I patients ($t=2.40$, $p < .02$) and with stage II patients ($t=3.79$, $p < .001$).

The presence of dyskinesia also significantly affected blinking ($F(2,77)=5.16$, $p < .01$, one-way ANOVA for independent measures). Dyskinetic patients had blink rates in excess of non-dyskinetics ($t=3.18$, $p < .01$) and normals ($t=1.97$, $p < .05$).

To examine the combined effects of dyskinesia and staging on patients blinking we used a two-way ANOVA for independent measures. The stage of parkinsonism ($F(1,48)=11.60$, $p < .01$) and the presence of dyskinesia ($F(2,48)=7.94$, $p < .01$) each affected blinking significantly, though these two variables did not interact significantly ($F(2,48)=1.15$, $p \geq .40$). Severely affected patients had decreased blink rates compared with other patients in both the dyskinetic ($t=2.63$, $p < .02$) and non-dyskinetic groups ($t=2.08$, $p < .05$). While the presence of dyskinesia was associated with increased blinking overall ($t=1.97$, $p < .05$), this effect was significant only in stage II patients ($t=3.17$, $p < .01$).

The constancy of the blink rate from one time period to another was tested by comparing blink rates from the two most recent counts in 31 patients in whom counts had been obtained previously on several occasions weeks apart. The mean blink rate for the first count was 17 ± 13 blinks/minute as compared with 18 ± 15 blinks/minute during the second count (intraclass correlation coefficient $=.58$, $p < .001$). The mean difference per patient was 7 ± 10 blinks/minute. The four patients were 2 standard deviations above the mean difference, accounting for more than half the total difference. If these patients are omitted from analysis, the mean difference for each of the remaining 26 patients decreases to 3 ± 4 blink per minute (intraclass correlation coefficient $=.86$, $p=.0001$).

Table 1

The Mean Blink Rates (blinks/minute) of Patients
Grouped by Stage and Dyskinetic Status

| Stage | I | II | III + IV | Overall mean \pm SD according to dyskinetic status |
|---------------------|----------------------|----------------------|----------------------|--|
| Dyskinetic Patients | 24 ± 14 (n=5) | 32 ± 14 (n=9) | 11 ± 11 (n=6) | 32 ± 16 (n=21) |

| | | | | |
|---|-------------------|-------------------|------------------|-------------------|
| Non-Dyskinetic Patients | 15 + 8 (n=18) | 13 + 11 (n=10) | 2 + 2 (n=5) | 12 + 10 (n=34) |
| Overall mean for patients in each stage | 17 + 10 (n=24) | 22 + 16 (n=19) | 7 + 10 (n=11) | |

C. Urinary Excretion in Parkinsonian Patients

Deprenyl is a selective inhibitor of monoamine oxidase type B (MAO-B) and has been reported to supplement the beneficial effects of L-DOPA in the treatment of parkinsonism. Chronic deprenyl intake elevates brain phenylethylamine (PEA), and gives rise to both amphetamine and methamphetamine in brain and urine. It is not clear, however, if the behavioral responses of chronic deprenyl treatment are related to the production of amphetamine and methamphetamine or to the inhibition of MAO or both. In a study of the effects of chronic deprenyl treatment on parkinsonian patients who were simultaneously treated with L-DOPA plus carbidopa, we analyzed the urine for the excretion of phenylethylamine (PEA) and other related biogenic amines. To evaluate the effects of deprenyl alone on the excretion of these compounds, we also analyzed urines from depressed patients after deprenyl treatment.

Urine was collected from five parkinsonian patients who were treated with Sinemet (L-DOPA plus carbidopa) before and after taking 10 mg (-) deprenyl daily for periods for 12 days to two months (Table 1). Urine was collected for 24 hours in bottles containing 10 ml 10% EDTA as a preservative. The volume was measured and aliquots were separated and stored at -40°C until analyzed. Urine was also collected from five control volunteers and a group of six depressed patients before and after taking 10 mg (-) deprenyl daily for one to four weeks. Biochemical determinations were performed "blindly".

Table 1

PATIENT DRUG SCHEDULES

| Patient number | Gender | Age (years) | Duration of deprenyl treatment | Daily dosage of deprenyl (mg) | Sinemet# | |
|----------------|--------|-------------|--------------------------------|-------------------------------|----------------|-------------|
| | | | | | Carbidopa (mg) | L-DOPA (mg) |
| 1 | male | 31 | 0 | 0 | 50 | 100 |
| | | | 63 days | 10 | 40 | 400 |
| 2 | female | 48 | 0 | 0 | 85 | 850 |
| | | | 48 days | 10 | 65 | 650 |
| 3 | female | 57 | 0 | 0 | 90 | 900 |
| | | | 12 days | 10 | 40 | 40 |
| 4 | male | 62 | 0 | 0 | 50 | 500 |
| | | | 56 days | 10 | 59 | 500 |

| | | | | | | |
|---|------|----|---------|---------|----------|------------|
| 5 | male | 54 | 55 days | 0 10 | 90 10 | 900 100 |
|---|------|----|---------|---------|----------|------------|

#Dose at the time of urine collection

Phenylethylamine, amphetamine and methamphetamine were measured by mass-fragmentography. Tyramine (both the m- and p-isomers), norepinephrine, dopamine, normetanephrine, and 3-methoxytyramine were measured by a direct method as follows: 20 μ l of urine was pipetted into 1 ml Microflex tubes, 10 ng of deuterated p-tyramine ($^2\text{H}_4$ -tyramine) in a volume of 10 μ l was added to all tubes, mixed and dried under a gentle stream of N_2 . The amines in the dried residue were derivatized with pentafluoropropionic anhydride to obtain the pentafluoropropionate derivatized. The metabolites of PEA and other amines, including the catecholamines, were measured. For the assay of p-tyramine, p-octopamine, and catecholamine acidic metabolites, 0.2 ml of urine was mixed into 1 ml of 1N HCl containing 100 ng of deuterated p-hydroxyphenylacetic acid, p-hydroxymandelic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and vanilmandelic acid (VMA). The metabolites were extracted, processed and derivatized. For 3-methoxy-4-hydroxyphenylglycol (MHPG), 0.2 ml of urine was mixed into 1 ml 1M acetate buffer (pH 6.2) and hydrolyzed at 40°C for one hour with 50 μ l crude sulfatase. This alcoholic metabolite was extracted and derivatized. All measurements of metabolites were carried out by comparing peak heights with those of appropriate deuterated isomers.

Finnigan models 3200 and 4000 combined gas-chromatograph quadrupole mass-spectrometers were employed (Table 2).

Table 2

MASS TO CHARGE RATIO (M/E) OF IONS FOCUSED UPON FOR THE
MASS-FRAGMENTOGRAPHY OF BIOGENIC AMINES, THEIR METABOLITES,
AMPHETAMINE, AND METHAMPHETAMINE

| | m/e | Deuterated isomer | m/e |
|-----------------------------------|-----|------------------------|-----|
| amphetamine (AMPH) | 118 | $^2\text{H}_6$ -AMPH | 123 |
| methamphetamine (M-AMPH) | 204 | $^2\text{H}_5$ -m-AMPH | 208 |
| phenylethylamine (PEA) | 104 | $^2\text{H}_4$ -PEA | 107 |
| phenylacetic acid (PAA) | 268 | $^7\text{H}_2$ -PAA | 275 |
| m-tyramine (m-ty) | 266 | | |
| p-tyramine (p-ty) | 266 | $^2\text{H}_4$ -p-ty | 269 |
| p-hydroxymandelic acid (PHMA) | 415 | $^2\text{H}_2$ -PHMA | 417 |
| p-hydroxyphenylacetic acid (PHPA) | 312 | $^2\text{H}_4$ -PHPA | 316 |

| | | | |
|---|-----|-----------------------|-----|
| vanilmandelic acid (VMA) | 445 | $^2\text{H}_2$ -VMA | 448 |
| 3-methoxy-4-hydroxy-phenylglycol (MHPG) | 622 | $^3\text{H}_2$ -MHPG | 625 |
| homovanillic acid (HVA) | 283 | $^2\text{H}_2$ -HVA | 285 |
| 3,4-dihydroxyphenylacetic acid (DOPAC) | 387 | $^2\text{H}_5$ -DOPAC | 392 |
| norepinephrine (NE) | 590 | $^2\text{H}_3$ -NE | 592 |
| dopamine (DA) | 428 | $^2\text{H}_4$ -DA | 431 |
| normetanephrine (NMN) | 458 | $^2\text{H}_3$ -NMN | 461 |
| 3-methoxytyramine (3MT) | 296 | $^2\text{H}_3$ -3MT | 299 |

Behaviorally, those patients who responded displayed less rigidity but also had signs of euphoria. Biochemically, amphetamine and methamphetamine were detected in measurable quantities in the urines of the parkinsonian and depressed patients after chronic deprenyl treatment. The excretion of methamphetamine ranged between 1 and 7 mg/24 hours. The ratio of methamphetamine to amphetamine ranged from one to three. We also analyzed urines from two parkinsonian patients eight and 15 days terminating deprenyl treatment. Both these deprenyl metabolites were detected (about 50 ug per 24 hours) indicating either that the metabolites of deprenyl or deprenyl itself or both, were slowly eliminated from the body.

Treatment of the parkinsonian patients with Sinemet (carbidopa plus L-DOPA) significantly increased the urinary output of PEA, m-tyramine and p-tyramine, with PEA showing the greatest change. The increase in PEA was not as great as that found in the depressed patients treated with deprenyl alone. Sinemet treatment of the parkinsonian patients failed to decrease the excretion of the major metabolites of PEA (phenylacetic acid) and p-tyramine (p-hydroxyphenylacetic acid).

To investigate the inhibitory effects of deprenyl on type A monoamine oxidase (MAO-A) activity, we measured the excretion of metabolites derived from p-octopamine (p-hydroxymandelic acid), p-tyramine (p-hydroxyphenylacetic acid), dopamine (3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and norepinephrine (vanilmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). These amines are all either good substrates for MAO-A or equally good substrates for MAO-A and MAO-B. Deprenyl, either alone or in combination with Sinemet, decreased VMA and MHPG ($p < .005$), but failed to reduce DOPAC and HVA excretion; Sinemet markedly increased the urine excretion of dopamine and 3-methoxy-tyramine.

Even though the number of parkinsonian and depressed patients studied was small, the excretion of p-tyramine and its metabolite, p-hydroxyphenylacetic acid, in the parkinsonians on Sinemet alone was significantly different from that observed in the normal controls and the depressed patients. Thus p-tyramine output in the parkinsonians was significantly lower than in the controls and depressed patients ($p < 0.05$). On the other hand p-hydroxyphenyl-

acetic acid excretion was higher in the parkinsonian patients than either the controls or depressed patients.

IV. Metabolic Disturbance

Platelet Monoamine Oxidase

The observation has been made previously that platelet, plasma and brain tissue monoamine oxidase activity is elevated in older individuals relative to younger subjects. This evident increase in enzyme activity with advancing age is in contrast to the other catecholamine enzymes. In addition to advancing age, certain pathologic states have been associated with elevated platelet monoamine oxidase activity including depressive disorders, senile dementia, and glaucoma.

Population studies of other catecholamine enzymes have demonstrated that very low enzyme activity is under genetic control, probably inherited as an autosomal recessive trait. Susceptibility to heat inactivation (thermolability) believed to be one of the most sensitive indicators of altered enzyme amino acid sequence, is under the control of the structural gene for the enzyme. Very low thermolability, which correlates with low enzyme activity in general population samples, is also evidently inherited as an autosomal recessive trait within families where both traits are present. Thermolability and low enzyme activity, however, sort independently for these other catecholamine enzymes.

Catecholamines in Postmortem Brains

Recent studies of cognitively impaired elderly point to an association between diminished central catecholamine function and dementia. While at last in part due to drugs, changes in catecholamine metabolism also have been observed centrally and peripherally in schizophrenic patients. Since dementia is frequently but not invariably seen in schizophrenia, we examined the relationship of peripheral catecholamine enzyme activity and cognitive function in a group of elderly schizophrenic patients and controls. Measurements were made of (1) plasma dopamine-beta-hydroxylase (DBH), the enzyme involved in the conversion of dopamine (DA) to norepinephrine (NE), (2) platelet monoamine oxidase (MAO) and (3) cognitive function measured by the Folstein Mini Mental State Exam (MMSE). Prior research indicates that after controlling for age both enzymes have an inverse relationship with MMSE scores. Since low MMSE scores are indicative of dementia, elevations of plasma DBH and platelet MAO activities were therefore associated with cognitive impairment in this sample.

From a total sample of 46 schizophrenic subjects and controls whose brains have been examined in post-mortem analysis for catecholamine concentrations in the hypothalamus and nucleus accumbens, a total of 20 subjects over the age of 60 identified (14 patients and six controls). All subjects histories were reviewed and examined the records to assess cognitive function using a modification of the Folstein MMSE (ante-mortem). Specifically, the records were examined for evidence that the subject was able to identify correctly and fully the date and their own location during the year prior to death. The records also were examined for evidence that the subjects demonstrated no evidence of impairment in either recall or remote memory; were able to read, comprehend, and write sentences; were able to perform serial 7's; and were able to comply with requests. None of the records contained information on the subject's ability to copy accurately two intersecting pentagons, so this standard item of the MMSE was omitted. Thus the maximum score on the modified MMSE was 31.

For all subjects measurements were made in the hypothalamus for DA, HVA, NE, and MHPG. In 19 of the subjects, the same catecholamine compound concentrations were measured in the nucleus accumbens. Dissection was performed at autopsy. Samples were stored at -50°C , then frozen in liquid nitrogen and pulverized. Random samples were taken for analysis by gas-chromatograph mass-spectrometry.

Certain bivariate relationships seen in the correlation matrix warrant mention prior to examining the results of the multiple regression analysis. Only HVA in the nucleus accumbens correlates with age ($r = -.66$, $p = .01$). The postmortem interval correlated significantly with MMSE scores ($r = -.58$, $p = .03$) reflecting the fact that all the demented subjects were institutionalized. Transfer from the hospital to the Medical Examiner's Office took longer than those cases that came directly to the Medical Examiners for autopsy. Since the postmortem interval did not correlate significantly with the concentration of any of the catechol compounds, it was omitted from the main effects models in the multivariate analyses. As might be expected from metabolic pathways, HVA correlated significantly with DA in both the hypothalamus and nucleus accumbens ($r = .59$, and $r = .53$, respectively). Finally, as will also be seen in the main effects models of the multivariate analysis, both DA and MHPG have inverse relationships with MMSE scores in the samples from the nucleus accumbens.

Table I and II show results from the multiple regression of MMSE on all catecholamine compounds, net of subject age, for both the hypothalamus and nucleus accumbens respectively. Equation 1 in each table removes the covariate effects associated with age and DA. Equations 2 through 4 (in Table I and II) are intermediate equations. The order of entry of the independent variables in these equations is similar to the previously identified sequence of the metabolic chains. The explanatory power associated with each additional variable is indicated by the squared multiple-partial statistic for each of the intermediate equations net of the preceeding ones. The last equation in each table is the complete main effects model. As shown in Table I, age does not have a significant effect on MMSE within this particular sample. This may be due to the older age of the controls ($T = 1.82$, $p = .08$). Furthermore, the controls had the best overall level of cognitive function of the groups compared ($T = 1.79$, $p = .08$). Although this sample does not demonstrate the usual indirect relationship of age with cognitive function, all regression coefficients pertaining to the effect of catecholamine concentrations on MMSE are to be interpreted net of age since these coefficients are, by definition, partial statistics. In the hypothalamus then, the primary finding is that poor cognitive performance is associated with reduced concentrations of HVA and MHPG. Furthermore, the contrasts contained in the dummy variables for diagnostic category DC_1 (nonschizophrenic psychotic patients vs controls) and DC_2 (schizophrenic patients vs controls) show that the schizophrenic subjects differ from normals. With respect to the relationship of the central catecholamines measured in the hypothalamus to MMSE, net of age, the contrast of the other psychiatric patients to normals is in the same direction although of borderline significance in the same direction ($p < .05$). The coefficients for the dummy variables indicate that the schizophrenic patients demonstrate significantly poorer cognitive function, controlling for the catecholamines measured in the hypothalamus, and are thus distinct from normals. Had the other psychiatric patient group been larger, it also may have been statistically distinguishable from the normals.

Since MHPG became significant and HVA remained significant with the addition of the dummy variables for diagnostic category, the hypothalamic data also were examined for the presence of interaction terms between diagnostic category and MHPG and HVA. None of the four interaction terms proved to be statistically significant. The absence of significant interaction terms indicates that the partial regression slopes are constant across all three

groups. The groups differ only with respect to intercepts of the regression line associated with diagnostic group membership.

Compounds analysis with the data collected from the nucleus accumbens, however, yield different results. As can be seen in Table II, both DA and MHPG have an inverse relationship with MMSE scores indicating that dementia is associated with elevated DA and MHPG concentrations in the nucleus accumbens. The diagnostic category variables did not prove to be significant for this location, nor did they contribute significantly to variance explained. Thus they have been omitted from Table II.

Table I

SELECT REGRESSION STATISTICS OF THE EFFECT OF AGE AND VARIOUS
CATECHOLAMINES ON MMSE SCORES: HYPOTHALAMIC MEASUREMENTS

| Independent Variables | Equation 1 | Equation 2 | Equation 3 | Equation 4 | Equation 5 |
|----------------------------------|------------|------------|------------|------------|------------|
| Age | .1907 | .4157 | .4217 | .4190 | .1719 |
| Dopamine | -.1733 | -.6738* | -.6297* | -.6232 | -.4501 |
| Homovanillic Acid | | .7258* | .7228 | .8592* | .7064** |
| Norepinephrine | | | .1934 | .0137 | .1448 |
| 3-Methoxy-4-Hydroxy-phenylglycol | | | | .3813 | .5208* |
| DC ₁₊ | | | | | -.4886 |
| DC ₂₊₊ | | | | | -.6216* |
| R ² | .05 | .22* | .21 | .47** | .68** |
| Multiple Partial R ² | --- | .18* | .0 | .32** | .40** |

*Significant at $p=.05$

**Significant at $p=.01$

+DC₁=Dummy variable contrasting non-schizophrenic psychotic patients with controls.

++DC₂=Dummy variable contrasting schizophrenic patients with controls.

Table II

SELECT REGRESSION STATISTICS OF AGE AND VARIOUS CATECHOLAMINES
ON MMSE SCORES: NUCLEUS ACCUMBENS MEASUREMENTS

| <u>Independent Variables</u> | <u>Equation 1</u> | <u>Equation 2</u> | <u>Equation 3</u> | <u>Equation 4</u> |
|----------------------------------|-------------------|-------------------|-------------------|-------------------|
| Age | -.0277 | .0621 | .0553 | .0077 |
| Dopamine | -.6751** | -.6761** | -.6766** | -.4571** |
| Homovanillic Acid | | .1351 | .1421 | .1602 |
| Norepinephrine | | | -.0465 | -.0751 |
| 3-Methoxy-4-Hydroxy-phenylglycol | | | | -.4381* |
| R ² | .38 | .46 | .46 | .60 |
| Multiple Partial R ₂ | — | .13 | .0 | .26* |

*Significant at p=.05

**Significant at p=.01

Data have now been collected from a general population study (N=460) of platelet monoamine oxidase activity in a demographically diverse sample without evidence of psychiatric illness (often associated with altered platelet monoamine oxidase activity). Within the entire sample, age correlates positively with enzyme activity. But no such relationship is present, however, in those subjects age 18-50 or those aged 51-79. The decade 50-59 shows the bulk of the increment in enzyme activity. In older individuals in this sample (age 50) there is a phenomenon of increased thermolability relative to younger subjects. This finding in the older subjects is opposite to the usual association of low enzyme activity and thermolability.

The total number of subjects has been increased in order to identify a subset of subjects with heat sensitive platelet monoamine oxidase. With the full complement of subjects, data from kinships with heat sensitive monoamine oxidase will be used to identify the mode of inheritance of this trait. We hope that normative data collected from this research will aid us in understanding the extent of these changes as well as identify potential genetic interplay with age effects in reference to platelet monoamine oxidase. At present, we continue to analyze the data and plan to have our final results in the next several months.

Further, we continue to assess data contained in the Health Inventory Survey for diseases that have been reported to be associated with alterations of platelet monoamine oxidase. Particularly, we are examining these data in terms of decreased activity as it relates to schizophrenia, diabetes and alcoholism and increased activity as it relates to dementia, Parkinson's disease, glaucoma and depression.

Data are compiled on all members of a household within 50,000 households. The data identified by kinship present the opportunity to observe any potential relationship between the prevalence of these diseases within the kinships relative to the prevalence of these diseases within the general population.

V. Cognition

Attention and Memory

The cognitive performance of older persons appears to be hampered by decreased memory and increased attentional deficits. It is held, generally, that the ability to perform these tasks in the human information processing system deteriorates throughout the normal aging process. It is observed, further, that older persons have difficulty ignoring irrelevant information. Information that is not needed but is not ignored hinders performance by occupying the processing resources that are already at a premium in the older person.

As a person becomes increasingly experienced at a task through extended practice, the need to devote attention to the task diminishes. The execution of the task becomes automatized. The processing of information underlying a task is composed of a collection of components, some of which are already automatic, some of which are not. One condition that promotes the automation of a task is the consistent mapping of each stimulus to a particular response.

The inclusion of both varied mapping and consistent mapping conditions in a study allows us to witness what happens when the demand for attention diminishes in one condition relative to another. These two conditions were employed in a memory search task. Subjects received approximately 2500 trials over 5 sessions. Seventeen subjects participated: 12 young people between the ages of 20 and 34 years and 5 older persons between the ages of 54 and 65 years. Search time (response time) and event-related brain activity for 4 scalp locations were recorded. The major purpose of the study was to determine how the change in attention allocation due to the development of automatic processing affected the event-related potentials (ERPs) in the two age groups.

The findings of greatest interest concern possible differences between ERPs for the consistent mapping and varied mapping conditions. It is apparent that an additional distinction should be made between Yes and No decisions. It is common to find longer response times to No decisions than to Yes decisions and this characteristic was observed in our work.

VI. Nerve Repair

It is considered axiomatic that complete severance of a peripheral nerve results in an absence of conduction across the gap, even if the nerve is repaired. Regardless of the method of repair, impulses initiated in the proximal stump of a severed nerve have not been recorded in the distal stump prior to restoration of the continuity of the fibers by regeneration. The purpose of our work is to determine if, and under what conditions, conduction can be obtained following reconnection of the stumps of a freshly transected peripheral nerve. Since last year's report, our research into nerve repair has expanded. One direction this research has taken has been to further investigate freezing procedures for preparation of severed nerve stumps. In the past, freezing was thought to allow better nerve preparation. This method was dropped because freezing techniques were not sufficiently sophisticated to

prevent deterioration of nerve tissue. In one set of experiments this past year new freezing techniques were employed resulting in a 75% survival rate. We are continuing experimentation to confirm this finding.

In another study, a method for evaluating sciatic nerve damage was developed from measurements of the prints of the hind feet of walking rats preserved on x-ray film. Four variables were measured from these tracks and comparisons between the damaged (experimental) and intact (normal) side were converted to percent deficits and averaged to obtain a "sciatic functional index" (SFI). The SFI was then measured under normal conditions, after nerve transection, nerve crush, and sham procedures. Reliability and repeatability of the SFI were found to be excellent. The effects of sciatic nerve transection and nerve crush as evaluated by this method agree very well with other methods of evaluating nerve damage. We propose that the SFI provides a simple, accurate, reliable, and repeatable method for evaluating the functional condition of the sciatic nerve in rats.

Another series of experiments were performed to test the possibility that potentials could be evoked in the distal stumps of recently severed and repaired nerves by electrical stimulation of the proximal nerve stumps. Under certain conditions, using the sciatic nerve of rats, such potentials were obtained in vivo in six of 51 nerves tested, as well as in vitro in four of 51 nerves. The characteristics of these responses suggested that they were not electrical or electromyographic artifacts. In rabbits, similar evoked potentials were obtained in vivo in seven of 40 nerves tested. Histological examination of these nerves suggested that successful transmission was obtained when there was an absence of extraneous material between the tips of the severed axons and, to some degree, when the tips of the axons from the repaired nerves were found to lie in close juxtaposition. We are beginning to think that electrophysiological studies such as these could be used experimentally to obtain an immediate evaluation of the quality of peripheral nerve repair methods.

In a final group of studies designed to test the previously described techniques of nerve stump protection and reconnection, we are experimenting with conditions simulating injury with a significant delay between the injury and the initiation of treatment. These experiments are in the beginning stages.

Significance to Biomedical Research and the Program of the Institute

The significance of our research into the problems of the aged is best seen in three areas; Parkinson's disease, senile dementia of the Alzheimer's type, and tardive dyskinesia. In light of the shift in our nation's demographics towards an older population, and the severity of these three conditions, increased understanding of these disease processes and new methods of treatment are critically needed.

Parkinson's disease, manifested primarily by abnormalities of movement and posture, is characterized by dopaminergic neuronal loss and gliosis in the brain. Current therapeutic approaches to Parkinson's disease involve administration of the drug L-dopa, a precursor of dopamine and dopamine-like agents. Despite some dramatic improvements, such therapeutic regimens are frequently not completely effective, or are associated with severe side effects. Many of these difficulties may result from, among other possibilities, the absence of the physiological mechanisms which normally regulate transmitter release from dopaminergic terminals. Our work grafting dopamine-producing cells into the brains of Parkinson model animals attempts to circumvent this problem by developing a technique that would allow a previously damaged brain to begin reproducing the necessary dopamine. In

developing this line of investigation intensive study has been generated internationally, leading to the first grafting operation in a human subject in Sweden.

Our examinations of the possible mechanisms involved in Alzheimer's disease are equally significant to the scientific community and the general population. Dementia is the major neuro-psychiatric disorder of old age. According to figures issued by the National Center for Health Statistics, organic brain syndromes afflict 58% of the more than one million Americans in nursing homes. Many senile dementia patients are housed in other chronic-care facilities such as state mental hospitals and Veterans Administration hospitals. More than half of the patients over age 65 in state and county mental hospitals also carry the diagnosis of chronic organic brain syndrome or senile dementia.

Senile dementia of the Alzheimer's type can be defined as progressive, age-related, chronic cognitive dysfunction. A number of hypotheses have been promulgated to explain the origins of the disease. One that has attracted much attention in recent years postulates an increased amount of aluminum in the brains of Alzheimer's patients. Our work into the possible role of aluminum, as well as our investigations of various potential drugs treatments, is timely and needed research.

Our work into the prevention and treatment of tardive dyskinesia, also, is of vital importance to both the medical and lay populations. Tardive dyskinesia is the most serious complication of long-term neuroleptic therapy. What was initially thought to be a rare clinical curiosity has become a significant public health hazard.

Typically, tardive dyskinesia occurs after years of neuroleptic administration. The syndrome consists of abnormal involuntary movements of the mouth and face, extremities, and trunk. The pathophysiology of tardive dyskinesia is not precisely understood and there is no satisfactory treatment. Our award winning investigations into this disorder have generated both significant findings as well as new research directions in many other laboratories.

Proposed Course

We plan to continue our work into the prevention and treatment of Parkinson's disease, senile dementia of the Alzheimer's type, and tardive dyskinesia. In our Parkinson's disease research our grafting work is expanding to include dopamine-producing tissue grafts into the brains of monkeys. In our Alzheimer's disease research we will be examining further the effects of sodium fluoride on the amelioration of the disease's symptoms as well as experimenting with potential pharmacological treatments. And in our tardive dyskinesia work, we will be examining the potential of various enzymes as biological markers to determine which patients are at highest risk to develop the disorder. By determining those most at risk, we should then be better able to develop treatment modalities that both control the patient's psychosis while reducing the risk that the patient will develop tardive dyskinesia.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01500-10 SMRP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Indolealkylamines and neuronal function | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">M. Economou-</td> <td style="width: 20%;">Guest Worker</td> <td style="width: 15%;">SMRP</td> <td style="width: 15%;">NIMH</td> </tr> <tr> <td></td> <td>Hadjiconstantinou</td> <td>Visiting Fellow</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td>Other:</td> <td>P. Panula</td> <td>Visiting Associate</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Z. Lackovic</td> <td>Guest Worker</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>P. E. Potter</td> <td>Chief</td> <td>SMRP-B</td> <td>NIMH</td> </tr> <tr> <td></td> <td>N. H. Neff</td> <td></td> <td></td> <td></td> </tr> </table> | | | PI: | M. Economou- | Guest Worker | SMRP | NIMH | | Hadjiconstantinou | Visiting Fellow | SMRP | NIMH | Other: | P. Panula | Visiting Associate | SMRP | NIMH | | Z. Lackovic | Guest Worker | SMRP | NIMH | | P. E. Potter | Chief | SMRP-B | NIMH | | N. H. Neff | | | |
| PI: | M. Economou- | Guest Worker | SMRP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Hadjiconstantinou | Visiting Fellow | SMRP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Other: | P. Panula | Visiting Associate | SMRP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Z. Lackovic | Guest Worker | SMRP | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | P. E. Potter | Chief | SMRP-B | NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | N. H. Neff | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Biochemical Pharmacology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 1.2 | OTHER: 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Our objective is to determine the role of <u>serotonin</u> in <u>spinal cord</u> and whether serotonin plays a role in <u>peripheral nerve function</u> . | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Our objective is to determine whether serotonin might play a role in the processing of information in the peripheral nervous system and to investigate its role in spinal cord function.

HPLC with electrochemical detection was used to assay the indoles. Immunohistochemistry with an antibody directed against serotonin was used to evaluate serotonin neurons in the spinal cord.

We found that the recently described serotonin-containing small intensely fluorescent (SIF) cells of superior cervical ganglion are, in part, modulated by preganglionic cholinergic neurons. For example, administration of the muscarinic agonists carbachol or oxotremorine increases the content of serotonin, and the increase induced by oxotremorine is blocked by atropine. Treatment with atropine alone or decentralization of the ganglion lowers the content of serotonin. Reserpine, p-chlorophenylalanine or fluoxetine treatment reduces the content of serotonin in the ganglion, suggesting that the SIF cell system has properties similar to serotonergic neurons of brain. We postulate that the serotonin containing SIF cells of the rat superior cervical ganglion participate in local circuit modulation of ganglionic transmission by receiving preganglionic information via muscarinic receptors.

All serotonin-containing nerve fibers in the cord have been assumed to originate in the midline raphe nuclei complex. In the brain, serotonin and substance P are often found together in the same neurons. We investigated whether serotonin might be found in the sensory ganglion which are known to contain substance P. We also evaluated the possibility that serotonin-containing interneurons might be present in the spinal cord. By chemical analysis we found the content of serotonin to be higher in the lumbar portion of the cord. Ventral horns contain more serotonin than dorsal horns. Ventral and dorsal roots contained about the same content of serotonin. Ten days after mid-thoracic transection of the cord there was a 95 percent decline of serotonin. After rhizotomy there was no change of serotonin in the cord. Of particular interest was the observation that there was a decline of serotonin in the ventral roots below a spinal cord transection, suggesting that serotonin axons might leave the cord via ventral roots. Our initial preliminary immunohistochemical studies revealed varicose fibers mostly in layer VIII, VII and X. There were terminal-like processes in layer II and scattered throughout the gray matter. Transection decreased immunoreactivity except in layer X and dorsal horns. Thus far only scattered mast cells were found in the nerve roots and sensory ganglia. From the information gained from these initial studies, we are testing the hypothesis that layer X contains serotonin interneurons whose fibers leave the spinal cord in the ventral roots. Our studies have provided evidence that serotonin containing neurons might modulate autonomic activity in sympathetic ganglia. They also suggest that some peripheral serotonin-containing nerves may originate from spinal cord.

Many of the drugs used to treat mental disorders modify the distribution and metabolism of brain serotonin. These drugs also induce side effects that are intolerable for some patients. Our objective is to provide new information about the neuronal systems that utilize serotonin as a transmitter so that we

will understand better their function in health and disease. Moreover, from a knowledge of the physiological role of serotonergic neurons we may be able to circumvent drug side effects.

Our future studies will deal with a more complete mapping of serotonergic neurons in the spinal cord and with the possibility of identifying serotonergic neurons that emerge from the spinal cord and innervate peripheral tissues. Should we identify peripheral organs that are innervated by serotonergic neurons, we will study the characteristics of the receptor system.

Publication:

Hadjiconstantinou, M., Potter, P.E., and Neff, N.H.: Transsynaptic modulation via muscarinic receptors of serotonin-containing SIF cells of superior cervical ganglion. J. Neuroscience, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01503-08 SMRP | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Pharmacology studies of acetylcholine turnover: Control of cholinergic pathways | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>D. L. Cheney</td> <td>Chief</td> <td>SMRP-M</td> <td>NIMH</td> </tr> <tr> <td>Other:</td> <td>J. Wroblewski</td> <td>Visiting Fellow</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>H. Thompson</td> <td>Psychologist</td> <td>SMRP</td> <td>NIMH</td> </tr> </table> | | | PI: | D. L. Cheney | Chief | SMRP-M | NIMH | Other: | J. Wroblewski | Visiting Fellow | SMRP | NIMH | | H. Thompson | Psychologist | SMRP | NIMH |
| PI: | D. L. Cheney | Chief | SMRP-M | NIMH | | | | | | | | | | | | | |
| Other: | J. Wroblewski | Visiting Fellow | SMRP | NIMH | | | | | | | | | | | | | |
| | H. Thompson | Psychologist | SMRP | NIMH | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | | | | | | | | | | | | | |
| SECTION Molecular Pharmacodynamics | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.7 | PROFESSIONAL: 0.4 | OTHER: 0.3 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Although increases in <u>plasma choline</u> cause some increase in brain tissue choline, there is no increase in <u>acetylcholine levels</u> or in <u>acetylcholine turnover rate</u> in any of the brain areas studied. Indeed, increased plasma choline reduced the turnover rate of acetylcholine in the <u>hippocampus</u> demonstrating that increasing the availability of choline does not increase the rate of acetylcholine synthesis. Subcutaneously injected apomorphine appears to have a biphasic effect on the turnover rate of acetylcholine. Lower doses appear to reduce the turnover rate of acetylcholine in the striatum whereas at higher doses the turnover rate returns to normal.</p> | | | | | | | | | | | | | | | | | |

Project Description:

The objective of this study was to improve our knowledge of the local circuitry regulating the cholinergic pathway.

To study turnover dynamics rats were infused with phosphoryl(^3H)choline and the tissue content of choline, acetylcholine and their deuterated analogs was determined using gas chromatography-mass fragmentography. From the percent incorporation of deuterium in acetylcholine and choline the fractional rate constant for acetylcholine efflux was calculated. The turnover rate of acetylcholine was obtained by multiplying the fractional rate constant by the steady-state content of acetylcholine.

In order to test whether increasing the availability of choline to rat brain increases the rate of acetylcholine synthesis in that tissue, the concentrations of choline and acetylcholine and the turnover rate of acetylcholine in striatum, hippocampus, and cerebral cortex were measured following changes in dietary choline, intraperitoneal choline, or intravenous infusion of choline. Increasing plasma choline caused some increase in tissue choline but did not increase acetylcholine levels nor acetylcholine turnover rate in any of the areas of brain studied. Indeed, in hippocampus, choline reduced the turnover rate of acetylcholine.

As a prelude to the study of which type of dopamine receptors may be involved in the observed effects of various dopamine agonists, a more detailed study of the effect of apomorphine on the turnover rate of acetylcholine in various areas of the brain was needed. Apomorphine given subcutaneously in doses ranging from 0.01 to 3.0 mg/kg body weight had no effect on the turnover rate of acetylcholine in the frontal or parietal cortex. There was a slight although non-significant decrease in the hippocampus. In the striatum the turnover rate of acetylcholine was decreased and a dose-dependency curve was obtained with the largest decrease at the dose of 0.2 mg/kg. At doses above 1.0 mg/kg the turnover rate of acetylcholine returned to the control values. Apomorphine in the striatum had no visible effect on the acetylcholine content. However, the total choline content was decreased at apomorphine doses affecting turnover. The following dopamine antagonists had no effect on the turnover rate in any of the brain regions studied: pergolide (0.5 mg/kg, s.c.; 60 min), 3-PPP (3.0 mg/kg, s.c., 60 min) and SKF 38393 (1.0 mg/kg, i.p., 60 min).

It remains unknown which type of dopamine receptors may be involved in the observed effects of apomorphine on the turnover rate of acetylcholine in the striatum. However, since the results may suggest a biphasic effect of this agonist, apomorphine may act at both postsynaptic and presynaptic levels. This may be further investigated by the use of dopamine agonists acting on specific dopamine receptors.

The septal-hippocampal cholinergic pathway appears to be involved in such associative processes as memory, learning, attention, motivation and sleep. By understanding the modulation of this important pathway it may be possible to elucidate the mechanisms whereby these associative processes are manifest. To this end studies are in progress to determine (1) if increased choline

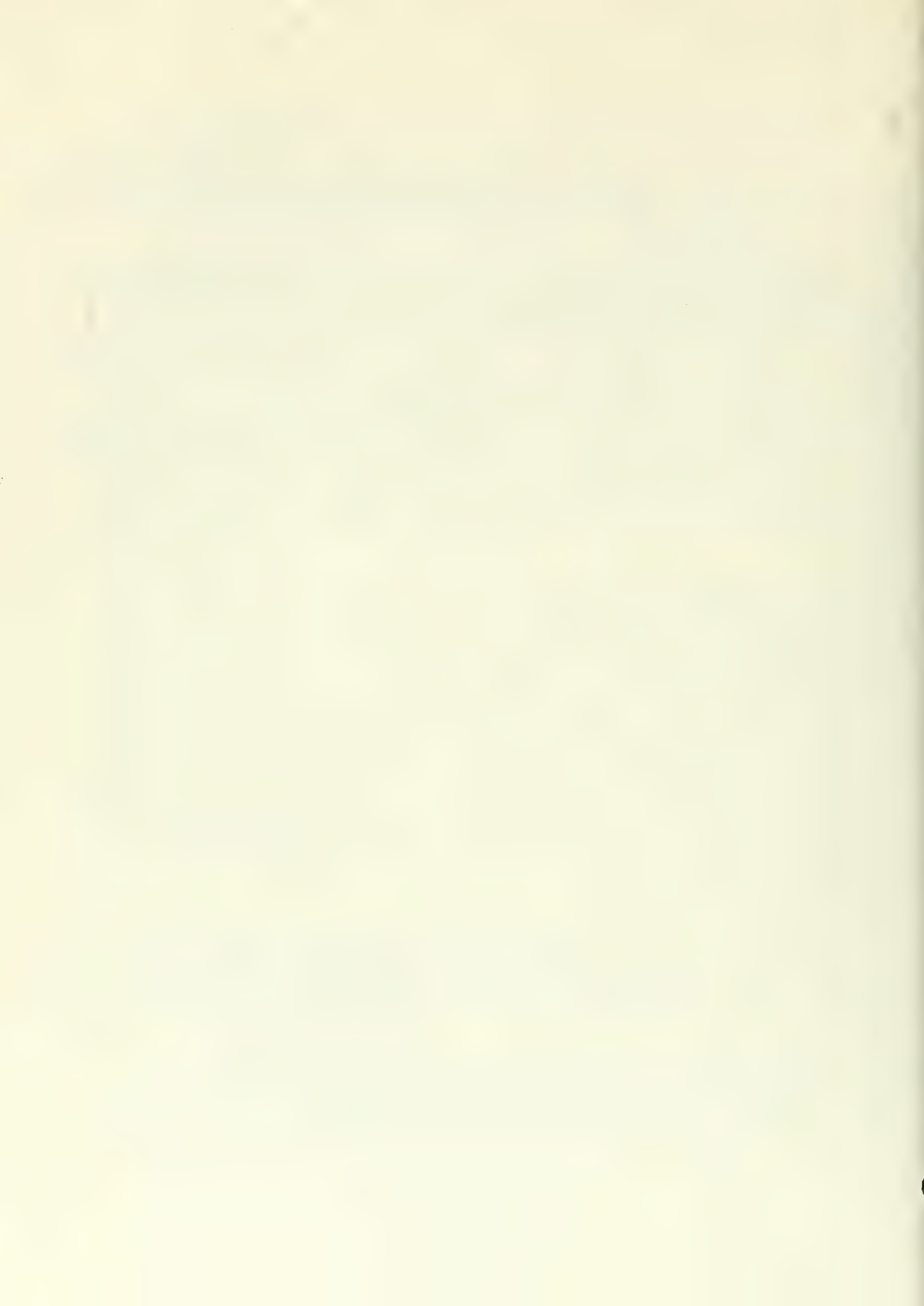
availability under conditions of increased cholinergic release increases the turnover of acetylcholine and (2) whether specific D_1 and D_2 dopamine receptors are involved in modulation of the septal-hippocampal cholinergic pathway.

Publications:

Brunello, N., and Cheney, D.L.: The septal-hippocampal cholinergic pathway: Role of antagonism of pentobarbital anesthesia and regulation by various afferents. J. Pharmacol. Exp. Ther. 219: 489-495, 1981.

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Cheney, D.L.: Drug effects on transmitter dynamics: An overview. In Hanin, I. (Ed.): Dynamics of Neurotransmitter Function. New York, Raven Press, in press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 01505-09 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Neurotransmitter dynamics: Chlordecone | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | O. Gandolfi M. Barbaccia D. L. Cheney | Guest Worker Visiting Fellow Chief |
| | | SMRP SMRP SMRP-M |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Pharmacodynamics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 1.1 | OTHER: 0.1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Adult male rats receiving a single i.p. injection of <u>chlordecone</u> (Kepone) (80 mg/kg) exhibited a decreased (³ H) <u>GABA binding</u> in frozen <u>cerebellar</u> and <u>hippocampal membranes</u> whereas the binding of (³ H)- <u>flunitrazepam</u> to cerebellar membranes was unaffected. The binding of (³ H)- <u>mianserin</u> was also depressed in hippocampal membranes whereas the (³ H)- <u>imipramine</u> binding was unaffected in any area studied. It is hypothesized that the study of alterations in cerebral high-affinity binding sites may be a useful tool to detect cerebral changes caused by Kepone. | | |

Project Description:

The objective of this study was to study neurotransmitter mechanisms which cause chlordecone toxicity.

Chlordecone (decochlorotetracyclodeconone) (Kepone) is a polycyclic chlorinated compound also known by the commercial name Kepone. Previous studies indicate that the neuronal system, the reproductive system, and the liver are major targets of Kepone toxicity. It has been found to cause neurotoxicity in man; these signs include tremors, headaches, abnormal elevation of cerebrospinal fluid pressure, mental symptoms, and visual disturbances.

Adult male rats (160-180 g) received a single i.p. injection (80 mg/kg) of Kepone and were killed 24 hr later. Rat brain regions were dissected and crude membranes were prepared. (^3H)-Imipramine (0.5-15 mM) binding assays were carried out at 0°C by incubating the radioactive ligand for 60 min with a crude membrane preparation. (^3H)-Mianserin (0.1-5.0 mM) binding assays were carried out at 37°C for 30 min with the same preparation. (^3H)-GABA (5-200 mM) and (^3H)-flunitrazepam (0.25-16 mM) binding assay were carried out at 0°C for 10 min. In the case of GABA and flunitrazepam assays, the final pellet was frozed and washed twice more in Tris-citrate (50 mM, pH 7.1) to assure removal of endogenous inhibitory materials. The incubations were carried out in the presence and in the absence of a large excess of a competing ligand. In all of the assays the specific binding was over 60% of the total binding.

In the hippocampus there was a 32% decrease in mianserin binding, an 18% decrease in GABA binding and a 35% decrease in spiroperidol binding. In the cerebellum there was a 34% decrease in GABA binding. Although the studies are not yet completed and there are binding studies yet to be finished in several of the tissues, there were no differences in binding by any of the ligands in any of the other tissues studied to date. Moreover, every change in binding is caused by a change in receptor density whereas the K_D is unaffected.

In accordance with the assumption that receptor density may indicate where neurotransmitter is released into the synaptic cleft, GABA mass-spectrometric and serotonin high performance-liquid chromatographic turnover studies are in progress.

The underlying mechanism of chlordecone toxicity is unknown. Since the receptor modulation may be an inescapable concomitant of neurotoxicity, both turnover and receptor studies could assist in the development of a objective screening procedure which can be used in the toxicological field. The underlying hypothesis of this research is that some neuronal circuits are more sensitive than others to the interaction with a toxicant. Therefore, broad screening procedures are essential if alterations of a transmitter or modulator system caused by a toxic agent are to be elucidated. In this report, the screening procedures focus on both the presynaptic and postsynaptic regions of the neuron. Future research will identify the specific alterations in the GABA and serotonergic systems caused by chlordecone.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01506-08 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Narcotic analgesics and the regulation of catecholaminergic neurons | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | L. Saiani A. Guidotti E. Costa | Visiting Fellow Chief Chief |
| | | SMRP SMRP-N SMRP |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Neuroendocrinology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS | | |
| <input type="checkbox"/> (b) HUMAN TISSUES | | |
| <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The role of opiate receptors located on catecholaminergic cells was studied using primary cultures of <u>adrenal chromaffin cells</u> . These cells contain opiate receptors in measurable amounts. Stimulation of these receptors with agonists decreases the release of catecholamines elicited by nicotine. This effect is stereospecific and is reverted by naloxone and dyrenorphine. The results suggest that stimulation of opiate receptors modulates allosterically the function of nicotine and other receptors located on the chromaffin cell membranes. | | |

Project Description:

Enkephalin-like peptides coexist in association with other neurotransmitters in many axons, including the splanchnic (cholinergic) nerve endings and the chromaffin cells of the bovine adrenal medulla. They are released from the latter in association with catecholamines. It is hypothesized that these enkephalin-like peptides act as cotransmitters or neuromodulators of the primary transmitter. Our objective was to study the modulatory role of opiates on the acetylcholine (ACh)-induced catecholamine release from cultured bovine adrenal medulla cells. These cells possess high affinity, stereospecific opiate binding sites; the addition of opiate receptor agonists to these cells inhibits the ACh-induced release of catecholamines, but not the release elicited by KCl or Ca^{++} ionophores. Thus the primary culture of adrenal chromaffin cells is an ideal model where to study the molecular mechanisms by which the narcotic analgesics control the function of catecholamine containing cells.

Results

For this study we have used various agonists with different affinities for μ , δ , κ and σ receptors and we have compared the same compounds for their ability to bind to adrenal membranes and for their potency to inhibit the ACh-induced release of catecholamines from chromaffin cells. 2 Etorphine, β -endorphin, met-enk[Arg⁶-Phe⁷] and the synthetic peptide [D-Ala², Me Phe⁴, Met(O)⁵-ol]-enkephalin inhibited the acetylcholine-induced release of catecholamines with an IC_{50} varying from 10^{-7} to 1×10^{-8} M. The effect was stereospecific because levorphanol ($\text{IC}_{50} = 7.5 \times 10^{-7}$ M) was approximately 2 orders of magnitude more potent than dextrorphan. Morphine (μ receptor agonist), [D-Ala²-D-Leu⁵]-enkephalin (δ receptor agonist), ethylketazocine (κ receptor agonist) and N-allylnormetazocine (σ receptor agonist) were at least 100-1000 times less potent than etorphine. Diprenorphine ($\text{IC}_{50} = 5 \times 10^{-7}$ M) and naloxone ($\text{IC}_{50} = 10^{-6}$ M) antagonized the effect of etorphine. High affinity, saturable and stereospecific binding sites for ^3H -etorphine, ^3H -dihydromorphine, ^3H -[D-Ala²-D-Leu⁵]-enkephalin, ^3H -ethylketazocine and ^3H -N-allylnormetazocine, ^3H -diprenorphine and ^3H -naloxone were detected in chromaffin cell membranes and in membranes obtained from adrenal medulla homogenates. However the number of binding sites for ^3H -etorphine and ^3H -diprenorphine was 10 to 70 times higher than the number of sites measured with the other ^3H -ligands. The rank order of potency of these compounds for the displacement of ^3H -etorphine binding correlates ($r=0.96$) with the rank order of potency of the same compounds for the inhibition of ACh-induced catecholamine release. These data suggest that a stereoselective opiate receptor (different from the classical μ , δ , κ or σ receptor) with high affinity for etorphine, diprenorphine, β -endorphin and met-enk[Arg⁶, Phe⁷] modulates the function of the nicotinic receptor in adrenal chromaffin cells.

Proposed Course

We intend to study the molecular mechanisms by which the stimulation of opiate receptors produce a decrease of acetylcholine-induced release of catecholamine from adrenal medulla cells.

Conclusions

The opiate receptors are present in membranes of adrenal chromaffin cells. Activation of these receptors causes a non-competitive inhibition of the release of catecholamines elicited by the stimulation of nicotinic receptors. From our data, it can be inferred that when the met-enkephalin-like material in terminals of splanchnic nerve is released, it modulates the release of catecholamines induced by the stimulation of nicotinic receptors elicited by the concomitant neurally mediated release of acetylcholine. Alternative modulation of adrenal nicotinic receptor can be achieved by opiate-like peptides from blood (i.e. β -endorphin) or from the adrenal cells themselves. The adrenal medulla contains several types of opioid-like peptides; they include met- and leu-enkephalin-like peptides, dynorphin 1-13, peptide E, met-enk[Arg⁶, Phe⁷], BAM 12P, BAM 20P and BAM 22P. Interestingly met-enk[Arg⁶, Phe⁷], a peptide present in high concentrations in adrenal medulla, is one of the most potent opiates tested. There is discussion whether this peptide acts by itself or after being converted to met-enk. Our data provide clear evidence that met-enk[Arg⁶, Phe⁷] is an opiate agonist on its own right because met-enkephalin and DADLE (a stable analogue of enkephalin) are two to three orders of magnitude less potent than the heptapeptide.

The interrelations between the opiate agonists and the nicotine-induced catecholamine secretion are relevant to the missions of the NIMH in many ways. The present study establishes a model to investigate how opiate receptors stimulation interact in the regulation of the activity of postsynaptic cells. On a more general ground these studies may help to learn the biological principles that regulate the functional interaction of a primary transmitter and coexisting neuropeptides in nerve axon terminal.

Publications:

Costa, E., Guidotti, A., Hanbauer, I., Hexum, T., Saiani, L., and Yang, H.-Y.T.: Regulation of cholinergic transmission in adrenal medulla. In Pepeu, G.C. and Ladinsky, H. (Eds.): Cholinergic Mechanisms. Plenum Publ. Corp., 1981, pp. 143-153.

Costa, E., Govoni, S., Guidotti, A., Hanbauer, I., Saiani, L., and Yang, H.-Y.T.: Enkephalin-like peptides as cotransmitters in splanchnic and adrenal medulla. Proc. Int. Symp. on Brain-Gut Axis. Florence, 1981, in press.

Saiani, L., and Guidotti, A.: Opiate receptor mediated inhibition of catecholamine release in primary cultures of bovine adrenal chromaffin cells. J. Neurochem., in press.

Project Description:

Our objective was to evaluate the effects of several neurotransmitter, neuromodulator, or neuroactive compounds on the dynamics of the cholinergic neurons in brain.

The turnover rate of acetylcholine was determined following an infusion of phosphoryl- ^3H -choline. The percent of incorporation of deuterium into the precursor, choline, and product, acetylcholine, was determined and the turnover rate was calculated by multiplying the fractional rate constant for acetylcholine efflux by the steady state concentration of this neurotransmitter.

Ethanol

The acute effects of oral administration of ethanol on the regional acetylcholine turnover on the brain were studied. After the administration of 5 g ETOH/kg body weight there was a rapid (10 min) decrease in the turnover rate of acetylcholine in the cortex, but no effect in the striatum or hippocampus. These changes were accompanied by sedation and persisted for at least 2 hours. However, this dose produced a drop in body temperature of approximately 2°C which was detectable 5 minutes after injection and lasted for at least 2 hrs. Maintenance of normal body temperature by radiant heat significantly reversed the effect on acetylcholine turnover in the cortex.

Adenosine

N^6 -Cyclohexyl [^3H]adenosine ([^3H]CHA) was used to label adenosine receptors in crude synaptic membranes prepared from rat and guinea pig brain. The density of [^3H]CHA binding sites was highest in the rat hippocampus and cerebellum, and in the guinea pig hippocampus. A microdissection of coronal sections of guinea pig hippocampus revealed that the specific binding capacity for [^3H]CHA in area CA-1 were 20-30% higher than in area CA-3, dentate gyrus, or subiculum. Selective neuronal lesions of serotonergic noradrenergic, and cholinergic afferents to the hippocampus failed to alter [^3H]CHA binding to hippocampal membranes. These results suggest that [^3H]CHA binding sites are not associated with axons and terminals of these neurons in the hippocampus. Intrahippocampal injection of kainic acid reduced the number of [^3H]CHA recognition sites by 30% with no alteration in the affinity of [^3H]CHA for these receptors. Thus, a significant portion of A_1 receptors may be associated with intrinsic neurons of the hippocampus which do not appear to be innervated by noradrenergic, cholinergic or serotonergic axons.

THIP

The subcutaneous administration of THIP (ED_{50} 3.0 mg/kg) to mice elicited a potent antinociceptive effect on the hot plate test. The onset of antinociceptive action occurred between 15 and 30 min post-injection and was maximal at 30 min; whereas, the duration was somewhat less than 60 min. Mice receiving up to 3 mg/kg of THIP subjectively evinced no obvious ataxia or reduction in spontaneous movement. After a dose of 4.5 mg/kg the animals appeared somewhat sedated in cages, but remained responsive to handling and were able to orient to vibrissal stimulation.

The analgesic effect of THIP was not antagonized by coadministration of subconvulsive doses of bicuculline. Treatment with bicuculline alone did not

significantly alter paw-lick latencies of either of these dosage levels. In addition, the administration of either naltrexone (2.5 and 10 mg/kg, i.p.) or diprenorphine (0.1 and 1.0 mg/kg, i.p.) failed to produce any significant alterations in the antinociceptive response to THIP. Interestingly, naltrexone treatment (2.5 mg and 10 mg/kg) appeared to antagonize the sedative effects of the 4.5 mg/kg dose of THIP. Mice injected with naltrexone plus THIP displayed an increase in locomotor activity, Straub tail and jumping behavior. This excitatory syndrome was never observed in mice treated with either naltrexone or THIP alone. A potentiation of the analgesic effect of morphine (2 mg/kg, s.c.) was observed in mice following treatment with THIP (1.5 mg/kg). This dose did not elicit any significant antinociceptive action when given alone.

In view of the demonstrated role of the descending bulbospinal serotonergic system in regulating nociceptive threshold, we examined the effects of pharmacological manipulations of serotonin function on THIP-induced analgesia. The administration of the putative serotonin agonist, MK-212, in a dose of 5 mg/kg elicited a significant analgesia response in the hot plate assay. Surprisingly, the intraperitoneal administration of metergoline, a serotonergic receptor antagonist, also produced a significant antinociceptive action when given in doses of 0.2 to 0.8 mg/kg. Doses of metergoline greater than 1.0 mg/kg were associated with a considerable amount of sedation, and hypoactivity characterized by a prone posture with limbs splayed out and an absence of movement following prodding. This high degree of motor impairment prevented the use of higher potentially nonanalgesic doses of metergoline in drug interaction studies. Treatment of mice with a subanalgesic dose of MK-212 (2.5 mg/kg) resulted in a significant reduction of the antinociceptive activity of both THIP (2 mg/kg) and morphine (2 mg/kg). The significant analgesic effects of low doses of metergoline prevented attempts to evaluate the influence of this serotonin antagonist on THIP and morphine induced antinociception.

Tolerance developed to the analgesia produced by THIP in mice treated three times daily for three consecutive days and tested on day four following THIP injection. It was not complete, however, since THIP (4.5 mg/kg) still produced a significant, albeit reduced, antinociceptive response in these animals.

These results suggest that the analgesic activity of THIP in the mouse hot plate test does not involve an interaction with opiate receptors, bicuculline-sensitive GABA receptors, or the recognition site for baclofen. Conversely, these results suggest that a selective activation of postsynaptic serotonergic receptors in the dorsal horn of the spinal cord may result in a potentiation of opiate or THIP-induced elevation in nociceptive threshold. Thus, the transsynaptic mechanisms by which THIP exerts its antinociceptive effects appear to be unique and may reflect an action on an unexplored neuronal system involved in the modulation of nociceptive threshold.

The identification of non-opiate mechanisms to produce antinociception may lead to the development of novel analgesics which have no tolerance or physical dependence liability. It is, therefore, important to continue these studies by investigating the transsynaptic mechanisms by which serotonin and GABA

agonists, respectively exert their antinociceptive effects in the spinal cord and brainstem.

Publications:

Murray, T.F., and Cheney, D.L.: The effect of phencyclidine on the turnover rate of acetylcholine in various regions of the rat brain. J. Pharmacol. Exp. Ther. 217: 733-737, 1981.

Murray, T.F., and Cheney, D.L.: Neuronal location of N⁶-cyclohexyl ³H-adenosine binding sites in rat and guinea pig brain. Neuropharmacology, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 MH 01509-12 SMRP | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | |
| TITLE OF PROJECT (80 characters or less) Psychopharmacological studies of acetylcholine turnover: Behavior | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| PI: W. D. Blaker | | Staff Fellow | | SMRP NIMH | |
| Other: D. L. Cheney | | Chief | | SMRP-M NIMH | |
| COOPERATING UNITS (if any) None | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | |
| SECTION Molecular Pharmacodynamics | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | |
| TOTAL MANYEARS: 1.1 | | PROFESSIONAL: 1.0 | | OTHER: 0.1 | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) That cholinergic activity in the <u>septal-hippocampal pathway</u> is involved in <u>extinction</u> was directly demonstrated by comparing the <u>acetylcholine turnover rate</u> (TR_{ACh}) in the rat <u>hippocampus</u> with <u>extinction</u> of a food reinforced lever press response after intraseptal injection of the GABA agonist <u>muscimol</u> . Doses (.3 to 3 nmoles) which decreased the TR_{ACh} also increased the response rate during extinction. Responding during the <u>continuous reinforcement</u> (CRF) schedule prior to extinction was also increased but to a lesser extent. Higher doses (10-30 nmoles) further decreased the TR_{ACh} and were accompanied by sedation. The TR_{ACh} in the hippocampus was also measured in drug-free rats undergoing extinction after training on a CRF or <u>variable interval</u> 60 seconds (VI-60) reinforcement schedule. Although the VI-60 rats responded more than the CRF rats during extinction, there were no differences between the TR_{ACh} s. These results indicate that <u>muscimol</u> -induced decreases in hippocampal TR_{ACh} are accompanied by interference with extinction, but that operantly-induced differences in this behavior are not accompanied by large changes in TR_{ACh} . We are currently investigating whether the relationship between TR_{ACh} and operant behavior after muscimol is causally related. | | | | | |

Project Description:

The present studies were undertaken to establish a behavioral correlate of pharmacologically-induced alterations in the turnover rate of acetylcholine in the hippocampus.

To measure cholinergic function phosphoryl($^3\text{H}_0$)choline was infused through the tail or jugular vein of rats and the incorporation of label into choline and acetylcholine was determined using gas chromatography-mass fragmentography. From the choline and acetylcholine curves representing the change with time of the incorporation of label the fractional rate constant for acetylcholine efflux could be calculated. The fractional rate constant multiplied by the steady-state content of acetylcholine determined the turnover rate of acetylcholine.

A variety of in vivo pharmacological manipulations of the medial septum in the rat brain have been shown to result in changes in the cholinergic activity of the septal-hippocampal pathway as measured by the turnover rate of acetylcholine in the hippocampus. For example, injection of the GABA agonist muscimol into the septum decreases the turnover rate of acetylcholine in the hippocampus in a dose dependent manner. Numerous studies have been done on the involvement of these limbic structures on various behaviors. Impairment of response inhibition, which can be measured by noting sustained responding during extinction, is induced by septal, hippocampal, or fornical lesions or by administration of anticholinergics to the hippocampus. This indicates that the cholinergic septal-hippocampal pathway plays a role in the mediation of response inhibition. Thus, the effect of intraseptal injections of muscimol on both the turnover rate of acetylcholine in the hippocampus and extinction of a food-reinforced lever press response in the rat were investigated.

Rats were implanted with chronic septal cannulae and trained on a continuous reinforcement schedule for several days. The animals were then injected with various amounts of muscimol via the septal cannula and 20 minutes later challenged with extinction of the learned response for 10 minutes. This was immediately followed by the infusion of phosphoryl($^3\text{H}_0$)choline into the tail vein for turnover rate determinations. It was found that the acute injection of muscimol decreased the acetylcholine turnover rate in the hippocampus and increased the responding during extinction when compared to saline-injected controls. The effects were parallel and dose-dependent up to 3 nmoles of muscimol but higher levels led to sedation. The muscimol administration also increased responding during continuous reinforcement, but to a lesser extent than during extinction.

Since pharmacologically-induced decreases in hippocampal acetylcholine turnover rate were accompanied by increased responding during extinction, the effect of operantly-induced increases in extinction responding on hippocampal acetylcholine turnover rate were investigated. Rats were trained on either a continuous reinforcement schedule or a variable interval -60 seconds reinforcement schedule. They were then implanted with chronic jugular vein cannulae and allowed to recover during additional training. The rats were challenged with extinction while at the same time being infused with phosphoryl($^3\text{H}_0$)choline for turnover rate determination. Although the variable interval

-60 seconds rats responded more than the continuous reinforcement rats, the hippocampal acetylcholine turnover rate was the same in these two groups and a third group which had received no training.

The mechanisms underlying the antianxiety nature of the benzodiazepines are still unknown. Therefore, these studies involving the interactions of benzodiazepines, behavior and neurotransmitter systems are an attempt to elucidate the mechanisms involved in antianxiety.

Future studies will be two fold: (1) receptor mechanisms involved in the apparent GABAergic influence of the turnover rate of acetylcholine in the hippocampus and extinction behavior will be studied pharmacologically using benzodiazepines and other GABAergic agents, (2) the neurotransmitter system(s) in the septum which function through GABAergic interneurons to produce both the biochemical and behavioral changes will be studied using pharmacological manipulations of the septum. These studies should yield information on the action of benzodiazepines on specified behaviors and on the role of defined neurotransmitter systems in the expression of specific aspects of the learning process.

Publication:

Brunello, N., Tagliamonte, A., Cheney, D.L., and Costa, E.: Effects of immobilization and cold exposure on the turnover rate of acetylcholine in rat brain areas. Neuroscience 6: 1759-1764, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01510-07 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Effect of cannabinoids on cholinergic and GABAergic dynamics in rat brain | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | A. V. Revuelta D. L. Cheney | Visiting Associate Chief |
| | | SMRP SMRP-M |
| | | NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Pharmacodynamics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.4 | PROFESSIONAL: 0.3 | OTHER: 0.1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Administration of the synthetic, crystalline cannabinoid, nabilone, and the potent dimethylheptyl derivative of (-)- Δ^8 -tetrahydrocannabinol reduce the turnover rate of acetylcholine in the hippocampus and reduce the turnover rate of GABA in the septum. As a working hypothesis we suggest that a group of inhibitory GABA-containing interneurons impinges on a second smaller group of inhibitory GABA-containing interneurons which modulates the activity of cholinergic cell bodies located in the medial septum whose long axons project to the hippocampus. | | |

Project Description:

The objective of this research project was to determine the effects of various cannabinoids on the dynamics of cholinergic and GABAergic neurons in the rat brain.

The turnover rate of acetylcholine was determined following an infusion of phosphoryl(^3H)choline and the turnover rate of GABA was determined following an infusion of ^{13}C -glucose. The percent of incorporation of deuterium or ^{13}C into the precursor and product was calculated. From the interrelationship between the precursor and product the fraction rate constant for either acetylcholine or GABA efflux was determined. The turnover rate was obtained by multiplying the fractional rate constant by the steady-state concentrations of the products.

Administration of the synthetic, crystalline cannabinoid, nabilone, and the potent dimethylheptyl derivative of $(-)-\Delta^8$ -tetrahydrocannabinol reduce the turnover rate of acetylcholine in the hippocampus and reduces the turnover rate of GABA in the septum. These events occur without altering the steady-state concentrations of either the precursors or the two putative neurotransmitters. A simple model in which cannabinoids transsynaptically activate inhibitory GABAergic septal neurons impinging on cholinergic septal neurons does not explain the data. As a working hypothesis we suggest that a group of inhibitory GABAergic interneurons impinges on a second smaller group of inhibitory GABA-containing interneurons which modulates the activity of cholinergic cell bodies located in the medial septum whose long axons project to the hippocampus.

Studies are in progress to determine if the GABA turnover rates are equally reduced in both the lateral and medial septum following administration of the cannabinoids.

One of the most selective and potent effects of the cannabinoids is their ability to reduce the turnover rate of acetylcholine in the hippocampus. This effect is modulated in the septum by GABA interneurons. The septal hippocampal pathway has been reported to be involved in such associative processes as memory, learning, attention, and motivation -- all of which are affected by the cannabinoids. By using the cannabinoids as tools and by understanding the mechanism whereby both turnover rates of GABA in the septum and acetylcholine in the hippocampus are reduced it may be possible to elucidate the underlying mechanisms of some of these associative processes.

Publications:

Costa, E., Cheney, D.L., and Murray, T.F.: Levonantradol-induced inhibition of acetylcholine turnover in rat hippocampus and striatum. J. Clin. Pharmacol. 21: 256S-261S, 1981.

Revuelta, A.V., and Cheney, D.L. Simultaneous reduction in the turnover rates of septal gamma-aminobutyric acid and hippocampal acetylcholine following administration of nabilone. Neuropharmacology 20: 1111-1114, 1981.

Revuelta, A.V., Cheney, D.L., and Costa, E.: The dimethylheptyl derivative of (-)- Δ^8 -tetrahydrocannabinol reduces the turnover rate of gamma-aminobutyric acid in the septum and nucleus accumbens. Life Sciences, in press.

Murray, T.F., Revuelta, A.V., and Cheney, D.L.: Modulation of cholinergic dynamics in the rat brain by levonantradol and Δ^9 -tetrahydrocannabinol. In Hanin I. (Ed.): Dynamics of Neurotransmitter Function. New York, Raven Press, in press.

Proposed Course:

This project has been terminated.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01512-09 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Transmitter interactions in the regulation of pituitary function | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. Guidotti Chief SMRP-N NIMH | | |
| COOPERATING UNITS (if any) L. Grandison, Dept. Physiology and Biophysics, College of Medicine and Chemistry, Rutgers Medical School, Piscataway, N.J. | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Neuroendocrinology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The role of <u>GABA</u> , <u>catecholamine</u> and <u>endorphins</u> in the response of <u>hypothalamus</u> to different psychoactive drugs was studied by monitoring pituitary hormone release. Endogenous opiates stimulate PRL and block FSH release by activation of hypothalamic opiate receptors. These opiate receptors may be located on cells containing prolactin releasing factors since median eminence DA neurons or serotonergic neurons apparently do not mediate the effects of morphine. Stimulation of hypothalamic or brain GABA receptors with <u>muscimol</u> fails to block morphine or haloperidol-induced PRL release. In contrast stimulation of GABA receptors in anterior pituitary prevents the morphine or haloperidol-induced PRL release. GABA receptors with characteristics similar to those of rat were observed also in human anterior pituitary. This suggests a possible physiological role of pituitary GABA receptors in the release of PRL. | | |

Project Description:

Hypothalamic neuronal mechanisms transsynaptically regulate behavior and secretion of polypeptide releasing factors (RF's). The RF's, in turn, regulate pituitary function by stimulating adenylate cyclase in the anterior pituitary. In order to define the type of neurotransmitters involved in the release of pituitary hormones, the effects of neuroactive drugs were monitored by the measurement of pituitary hormone release.

1) Effect of opiate receptor agonists and antagonists

Injection of β -endorphin into the basomedial hypothalamus induced an increase of PRL release. Naltrexone was able to block this response. Specificity was further demonstrated since a 10-fold higher dose of γ -endorphin (inactive peptide) was ineffective. When injected into the hypothalamus naltrexone itself produced a suppression of PRL release. After deafferentation morphine still stimulated and naltrexone still inhibited PRL release. In contrast to its activity in vivo, β -endorphin (2×10^{-8} M; 2×10^{-7} M) or naltrexone (10^{-6} M) had no effect on PRL release from the pituitary in vitro. In addition, β -endorphin was unable to alter the inhibitory action of 5×10^{-7} M apomorphine on in vitro pituitary halves.

Next, we attempted to determine the events that mediate the PRL release elicited by stimulation of hypothalamic opiate receptors. When the DA tonus was potentiated by giving pargyline (a monoaminoxidase inhibitor), PRL release following submaximal doses of morphine or β -endorphin was not altered. In contrast, when the DA activity was reduced by administration of reserpine or haloperidol, pargyline significantly reduced the increase of PRL elicited by opiate receptor stimulation. In addition, the action of morphine or β -endorphin but not that of reserpine or haloperidol was blocked by naltrexone.

2) Effect of GABA receptor agonists

The rise in serum prolactin (PRL) concentration after haloperidol (0.05 to 0.5 mg/kg i.v.) or morphine (2 to 16 mg/kg s.c.) in male rats and the PRL increase during estrogen treatment (1 mg/day/15 days) to ovariectomized rats was reduced by systemic injection of the GABA receptor agonist muscimol (1 mg/kg i.v.). However, intraventricular or intrahypothalamic injection of muscimol (100-400 ng) was ineffective in blocking PRL release induced by these compounds.

The concentration of muscimol in hypothalamus after local injection was similar to that obtained following systemic injection of high doses of muscimol. Thus it appears that stimulation of central GABA receptor was not involved in the regulation of PRL release by i.v. muscimol: After this treatment the muscimol content in the anterior pituitary is high whereas the drug is almost absent in the pituitary of rats receiving muscimol intrahypothalamically (100-400 ng). These considerations have suggested that blockade of PRL release by muscimol is due to a stimulation of GABA receptors located in the pituitary. Indeed, 3 H-muscimol and 3 H-GABA receptor recognition sites with characteristics similar to those of brain were present in rats anterior pituitary. In addition, muscimol (10^{-8} to 10^{-6} M) reduced the spontaneous release of

PRL from anterior pituitary halves incubated, in vitro. This effect was reversed by the GABA receptor antagonist, bicuculline, but not by the dopamine antagonist, sulpiride. These results provide evidence that GABA receptor stimulation in the anterior pituitary can act to regulate the release of PRL.

From these studies it appears that different neurotransmitter systems are involved in the release of specific anterior pituitary hormones. For example, PRL release is under hypothalamic DA and endorphin control but not under a direct hypothalamic GABA control. GABA might control PRL release acting directly at the pituitary level independently from catecholamines. Very recently, we observed that GABA receptors are present in human anterior pituitary. Further efforts are now required to extend these studies to the numerous hypothalamic peptide neurotransmitter substances (neurotensin, somatostatin, substance P, bombesin) which have been shown to influence hormone release. These observations and the demonstration of large concentrations of GABA in human anterior pituitary suggest a possible physiological role of pituitary GABA receptors in the control of PRL release. In addition, if PRL release can be controlled by stimulation of GABA receptors located on the anterior pituitary, peripheral GABA-mimetic drugs that do not enter the brain may represent a relevant approach to the control of the PRL releasing actions of neuroleptics without altering their central effects.

In future studies we plan to establish the anatomical localization of GABA, GABA receptors and benzodiazepine receptors. In addition further studies are required to elucidate the relationship between GABA and benzodiazepine receptors in human anterior pituitary tissue.

These studies are relevant to understanding total brain function because the hypothalamic neuronal peptides investigated here are known to be widely distributed throughout the brain. The hormone release neurotransmitter function relationship in the hypothalamus-pituitary axis may be used as a tool to learn the action of different psychoactive drugs on neuropeptide transmission.

Publication:

Grandison, L., and Guidotti, A.: GABA and benzodiazepines binding sites in human anterior pituitary tissue. J. Clin. Endocrinol. Metab. 54: 597-601, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01514-10 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Trans-synaptic control of protein synthesis | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | L. Saiani A. Guidotti E. Costa | Visiting Fellow Chief Chief |
| | | SMRP SMRP-N SMRP NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Neuroendocrinology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.3 | PROFESSIONAL: 0.3 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) In primary <u>culture of chromaffin cells</u> from cow adrenal medulla TH induction is preceded by an activation of <u>cytosol cAPK</u> and by an increase in nuclear protein phosphorylation. Both the induction of TH and the increase of nuclear phosphorylation require that the assembly of <u>microtubular proteins</u> be functional. Anti-microtubular drugs such as <u>colchicine</u> and <u>vinblastine</u> (10^{-9} M) can block the TH induction elicited by 8-Br-cAMP, when the drugs are added less than 15 hours after 8-Br-cAMP. Since colchicine, added within cAMP also prevents the increase in nuclear phosphorylation, it is possible that the assembly of microtubular proteins might be operative in the <u>intracellular translocation</u> and nuclear uptake of catalytic subunits of <u>cAPK</u> activated by the addition of 8-Br-cAMP. In addition, these data support the view that an increase in nuclear protein phosphorylation is an essential step in the mediation of the acceleration of mRNA synthesis and the subsequent increase in TH synthesis elicited by 8-Br-cAMP. | | |

Project Description:

Our objective was to study the molecular mechanisms whereby transsynaptic stimuli induce new synthesis of specific proteins in chromaffin cells of adrenal medulla. In previous studies we reported that in chromaffin cells of rat adrenal medulla, the sequence of molecular events whereby transsynaptic mechanisms regulate the genetic code expression includes an increase in the cAMP/cGMP concentration ratio, an activation of cAMP-dependent protein kinase (PK) in cytosol and the translocation of the low molecular weight catalytic subunit of this protein kinase from the cytosol to the subcellular particles. The PK of nuclei is not regulated by cAMP but it increases during the transsynaptic induction of tyrosine-3-monooxygenase (TH) because the cAPK catalytic subunits translocate from cytosol to the nucleus. Thus, the activation and translocation of PK, triggered by the initial increase of cAMP, acts as a long range messenger for the transsynaptic expression of the genetic code.

An injection of nicotinic receptor antagonist or the denervation of adrenal medulla reduces and shortens the cAMP elevation caused by cold exposure and prevents the cAPK activation: it inhibits the nuclear translocation of cAPK catalytic subunits and also blocks the induction of TH. Though these experiments demonstrate a temporal succession between the translocation of the catalytic subunits of cAPK to the nucleus, the increase in nuclear protein phosphorylation and the stimulation of gene activity leading to an increase in TH synthesis, they fail to elucidate: 1) the specific regulatory mechanism involved in the nuclear uptake of catalytic subunits of cAPK; 2) the precise role in nuclear function of the subunits of cAPK that translocate to the nucleus; 3) the mechanisms underlying gene transcription specificity; and 4) the necessity of nuclear translocation of cAPK catalytic subunits in eliciting the transsynaptic induction of TH.

In vivo experiments with rat adrenal medulla are not the most suitable means to study transsynaptic regulation of gene expression at the molecular level because rat adrenal medulla provide only a limited amount of purified nuclear material which does not allow a rigorous monitoring of the fine biochemical events occurring in the nuclear chromatin.

We have then decided to use as an in vitro system primary cultures of cow adrenal medulla cells. These cells were used as a model to evaluate the ability of 8-Br-cyclic AMP (8-Br-cAMP) to induce TH and to study the role of cAPK in this induction. This cell culture maintains a constant level of cyclic nucleotides, catecholamines and related enzyme activities for about four weeks.

Exposure of the cells for 5 hrs to 8-Br-cAMP produces 48 hrs later, a dose related longlasting increase in TH activity; 8-Br-cGMP fails to modify TH. The increase in TH activity caused by 8-Br-cAMP is due to an increase of the V_{max} and is preceded by an activation of cytosol cAPK associated with a decrease of the total cytosol cAPK. A sustained increase in nuclear phosphorylation begins 8 to 12 hrs after 8-Br-cAMP application. The delayed increase in TH activity induced by 8-Br-cAMP is blocked by actinomycin D, cycloheximide, colchicine and vinblastine. This reduction of TH induction elicited by colchicine and vinblastine (10^{-9} M) is observed only when these inhibitors of the microtubular

protein polymerization were added 4 to 12 hrs after the addition of 8Br-cAMP which is the inducing stimulus. The addition of colchicine 15 hrs after 8-Br-cAMP fails to inhibit TH induction. This blockade of TH induction is associated with an inhibition of the increase in nuclear phosphorylation, but is not associated with an inhibition of protein synthesis. The increase of endogenous cAMP and the induction of TH were also produced by cholera toxin. These results suggest that the increase of TH elicited by 8-Br-cAMP is mediated by the translocation of cAPK subunits from cytosol to the nuclei and that this translocation requires the function of the microtubular network.

In future studies, we plan to study how the increase in nuclear phosphorylation regulates the expression of the genetic code during the induction of TH. Measurement of turnover of mRNA coding for TH should be studied using specific complementary DNA that hybridize with the respective mRNA sequence.

Since in adrenal medulla, the availability of catecholamines and the adrenergic function are regulated by TH and since adrenergic mechanisms have been implicated in the etiology of affective disorders an understanding of the molecular nature of the regulation of the biosynthesis of catecholamines may contribute to a better understanding of the synaptic defects that may be operative in the etiology of mental diseases. In addition, the translocation of cAPK subunits from the cytosol to the nuclei may operate as a basic mechanism in memory and/or learning.

Publication:

Guidotti, A., Chuang, D.M., Kumakura, K., and Costa, E.: Molecular biology of tyrosine hydroxylase induction. In Usdin, E., Weiner, N., and Youdim, M.B.H. (Eds.): Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects. London, MacMillan, 1981, pp. 141-147.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01515-09 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Short and long term biochemical changes in cerebellum after treatment with psychoactive drugs | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | D. S. Shah J. P. Chambon A. Guidotti | Visiting Associate Guest Worker Chief |
| | | SMRP SMRP SMRP-N |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Neuroendocrinology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.3 | PROFESSIONAL: 1.3 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Biochemically, the <u>cerebellum</u> is characterized by a large concentration of <u>cGMP</u> , <u>cAMP</u> , and <u>cGMP-dependent protein kinase</u> . Drug-induced changes in <u>GABA receptor</u> function can be easily monitored by measuring changes in these biochemical parameters. It is suggested that diazepam and muscimol (a modulator and a direct GABA receptor agonist, respectively), modulate cerebellar function by altering the cGMP system. Diphenylhydantoin has its own receptor on cerebellar structures and this receptor is modulated by benzodiazepines. We are now investigating short and long term biochemical changes associated with the stimulation of diphenylhydantoin, receptors and the interrelationship of this receptor with the GABA-benzodiazepine receptor complex. | | |

Project Description:

In previous reports we have shown that pharmacological manipulation of climbing or mossy fibers not only change the firing pattern of Purkinje cells but also increase the cGMP content of the cerebellar cortex. Similarly, manipulation of the cerebellar GABAergic function changes the firing rate of the Purkinje cells and the cerebellar cGMP content. When the concentration of cerebellar GABA is increased or GABA receptors are stimulated, the firing rate of Purkinje cells and the cGMP content of cerebellar cortex are reduced. Conversely, when GABA receptors are blocked, the firing rate of Purkinje cells and the cerebellar cGMP content are increased.

Since diazepam modulates cerebellar cGMP content by regulating GABA receptor excitability, we have decided to test whether another anticonvulsant such as diphenylhydantoin which is chemically related to diazepam, was acting on the cGMP-diazepam-GABA interactions operative in cerebellum. As first approach to this problem we have decided to study the binding characteristics of ^3H diphenylhydantoin (DPH) to crude synaptic membranes prepared from rat brain.

The binding of ^3H -DPH was measured either in fresh P-2 brain synaptosomal membranes or in frozen-thawed and repeatedly washed membrane preparations. Using 50 mM Tris-HCl, 100 mM NaCl, pH 7.4, ^3H -DPH binding was determined by filtration technique using GF/C filters in presence and absence of 2.10^{-5}M cold DPH. ^3H -5,5-Diphenylhydantoin (DPH) specifically binds to a high affinity site which is present in rat brain; this site is thermostable. This high affinity site ($K_d=14\text{ nM}$) has a low binding capacity (B_{max} of 50 fmol/mg prot). Brain contains another population of sites which binds ^3H -DPH with low affinity ($K_d=700\text{ nM}$) and high B_{max} (2.1 pmol/mg prot). The specific binding of ^3H -DPH is inhibited by cold DPH, ethothoin, spirodilantin and pentobarbital but it is enhanced by diazepam. Trypsin and pronase decreased the specific binding of ^3H -DPH while phospholipase C and D, Ca^{++} , EGTA failed to modify the binding of ^3H -DPH. The B_{max} for the high affinity binding sites is highest in cortex and cerebellum. The B_{max} value of spinal cord was about 1/3 that of cortex. Binding of ^3H -DPH was also found in kidney, heart and lung however the number of sites per unit of protein is smaller than in brain.

Prior exposure of crude synaptic membranes to $5 \times 10^{-6}\text{M}$ concentrations of diazepam, flunitrazepam, clonazepam, chlordiazepoxide and medazepam increased the specific binding of ^3H -DPH by a varying extent (from 36 to 167 percent). Diazepam was the most potent (167 percent) and its effect was dose related. In heat treated membranes and non-neuronal tissue such as liver, diazepam failed to increase the specific binding of ^3H -DPH. Under the experimental conditions used the improvement of ^3H -DPH specific binding by diazepam, chlordiazepoxide and medazepam is related with their pharmacological potency. However flunitrazepam and clonazepam failed to be more potent than diazepam. Ethyl- β -carboxylate which displaces diazepam from its binding sites very effectively failed to modify the diazepam induced facilitation of ^3H -DPH binding. However it is relevant in support for a specificity of action that the diazepam facilitation of ^3H -DPH binding fails to occur in the liver. It is of interest to note that s-adenosyl methionine (a methyl donor) potentiated the diazepam induced facilitation of ^3H -DPH binding (Unpublished observations), suggesting that diazepam may act by modifying membrane phospholipids composition.

Future studies will be focussed on the effect of benzodiazepines (acute or chronic) on phospholipid methylation and composition of membranes in neuronal cells in culture. If changes will be observed these changes will be related to modification of ^3H -DPH binding and DPH effects.

Considering that high affinity binding sites for drugs have the potential of revealing the presence of endogenous ligands which modulate neuronal function the studies have the possibility of increasing our understanding of interaction of neuromodulator and primary transmitters. This type of interaction is particularly interesting to neuropharmacology because has been shown to lead to development of neuroactive drugs with low toxicity.

Publication:

Shah, D.S., Chambon, J.P., and Guidotti, A.: ^3H -Diphenylhydantoin binding to rat brain membrane. Neuropharmacology 20: 1115-1119, 1981.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01516-09 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biochemical pharmacology of minor tranquilizers | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | C. M. Forchetti A. Guidotti D. Konkell E. Costa | Guest Worker Chief Chemist Chief |
| | | SMRP SMRP-N SMRP SMRP |
| | | NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Neuroendocrinology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 0.7 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Behavioral studies have shown similarity of action between <u>benzodiazepines</u> (BDZs) and facilitation of GABAergic transmission, indicating that the pharmacological action of BDZs is mediated through GABAergic synapses. The molecular composition of the GABA receptors was studied biochemically. Two separate binding sites (one for ³ H-GABA and one for ³ H-diazepam) were isolated by differential solubilization from rat brain homogenates with Triton X-100. Another element of the GABA receptor complex has been isolated, an <u>endogenous brain peptide</u> (DBI) that probably represents the endogenous ligand for the BDZ receptor. DBI was prepared from rat brain by extraction in hot 1N acetic acid and was purified to homogeneity by Sephadex and ion exchange chromatography and by reverse phase HPLC. Purified DBI contains 10 amino acid residues and is basic in nature. This material inhibits competitively ³ H-diazepam and ³ H-beta-carboline. Behavioral and binding studies indicates that DBI is an effector for the benzodiazepine receptor with similarities to the anxiogenic β -carbolines. These data suggest that GABA receptors and BDZ receptors are functionally associated and that an endogenous peptide regulates their function. | | |

Project Description:

Recent evidence from this laboratory has suggested that benzodiazepines, may exert a beneficial effect in psychiatric disturbance by a primary action on GABAergic transmission. The present project intends to elucidate the molecular mechanisms of action of benzodiazepines (BDZs).

In order to elucidate this problem we have decided to initiate studies devoted to the solubilization and partial purification from rat brain cortex homogenates of [^3H] γ -aminobutyric acid (GABA) and [^3H]diazepam recognition sites and of their endogenous modulators (GABA-modulin and an endogenous compound which displaces specifically bound [^3H]diazepam).

Results

A high percentage of GABA binding sites (virtually free of benzodiazepine binding sites) was solubilized from homogenates of rat brain cortex incubated at 0°C with 1% Triton X-100 and a mixture of protease inhibitors. A large proportion of benzodiazepine binding sites was solubilized in the absence of apparent GABA binding capacity by incubating crude synaptic membrane preparations at 37°C with 0.05% Triton X-100. The characteristics of these two solubilized binding sites resemble those of the membrane-bound binding sites. However, unlike the membrane-bound sites, solubilized GABA and benzodiazepine recognition sites have lost the ability to cross-react. Hence, solubilized benzodiazepine binding sites are insensitive to GABA stimulation, while solubilized GABA binding sites are no longer protected by the benzodiazepines against heat inactivation. These results indicate that GABA and benzodiazepine recognition sites reside in two different molecules which, when bound to membranes, can interact reciprocally and modulate their binding affinity for specific ligands.

The ligand for the benzodiazepine receptor is probably represented by an endogenous brain peptide (DBI) that we have isolated and partially characterized. The extraction procedure was initiated by homogenizing the rat brain in 20 vol of hot (80°) 1 N acetic acid. After the pH was adjusted to 5, the 48,000 xg supernatant was chromatographed in succession on Sephadex G-100, Sephadex G-75, Biogel P₂, HPLC Synchronapak AX 300 anion exchange and HPLC reverse phase Bio-Sil ODS-10 column. Furthermore impurities of GABA-modulin which copurify with DBI can be removed with a 60% ammonium sulphate precipitation. Purification of DBI was achieved by rechromatography on a reverse phase Bio-Sil ODS-10 HPLC column using as mobile phase $0.1\text{ N NaH}_2\text{PO}_4 + 0.1\% \text{ H}_3\text{PO}_4$ pH 2.5 and 0-60% acetonitrile gradient as eluant.

The protein associated with DBI activity was retained by the reverse phase column and was eluted as a single sharp peak with high concentrations of acetonitrile (approx. 55%). Trypsin, as well as chymotrypsin and pronase, degraded the protein yielding several peptides. None of these peptides retains the DBI activity.

The material eluted from HPLC is virtually homogenous as determined by SDS gel electrophoresis and by HPLC using different methods and columns. N-Terminal amino acid analysis revealed that this group is blocked in purified DBI

preparations, in contrast the carboxy terminus is free and is probably tyrosine.

Using ^{125}I -DBI we have been able to measure the recovery of DBI throughout the purification. Overall, only 1-2% of the original amount of DBI present in brain was recovered in purified form. Since from a rat brain we recovered 2.5-5 μg of pure DBI, it can be calculated that the total amount of DBI in a brain is 0.25-0.5 mg. Furthermore, the molecular weight of DBI estimated both by measuring the amino acid residues or by SDS gel electrophoresis is around 10,000 daltons. Thus, it can be calculated that DBI concentration in rat brain is approximately 10-25 μM . We have not yet established the distribution of DBI in the brain, but we know that DBI can not be detected in extracts from liver, kidney and spleen of rats.

DBI prevents binding of several specific ^3H -ligands to the BZD recognition site present in synaptic membranes of rat brain. Doses of DBI which inhibited the binding of ^3H -diazepam, ^3H - β -carboline ethylester (β -CCE), ^3H -flunitrazepam or ^3H -RO 15-1788 by more than 50% failed to inhibit ^3H -morphine, ^3H -adenosine, ^3H -QNB, ^3H -isoproterenol and ^3H -imipramine binding.

The mechanism of the inhibition of ^3H -diazepam binding appears to be competitive. The K_i of DBI to displace ^3H -diazepam binding from crude synaptic membranes is approximately 1 μM . One important question to be addressed is whether DBI bound to the BZD recognition sites determines conformational changes of the receptor similar to those elicited by the benzodiazepines or resembles those elicited by the beta carbolines analogues endowed with anxiogenic or proconvulsant properties. To answer this question, the most direct approach was to test how DBI acts in behavioral tests which can predict the anxiolytic or anxiogenic effects of a compound. Rats receiving DBI intracerebroventricularly (50-100 μg) fail to show any obvious change in behavior. However, DBI (50 μg i.c.v.) reduced the anticonflict action of diazepam on the shock induced suppression of drinking by thirsty rats.

Another approach to establish whether DBI is similar to either diazepam or to β -CCE is to test how DBI modifies or displaces the ^3H - β -CCE or the ^3H -benzodiazepines bound to the membranes. When the binding of ^3H -RO 15-1788 to synaptic membranes was displaced by diazepam or DBI or β -CCE in the presence or in the absence of GABA it was observed that GABA potentiated the effect of diazepam while that of DBI and β -CCE was not. In another group of experiments DBI and β -CCE were more effective in displacing ^3H -flunitrazepam from the cerebellum than from the hippocampus. Finally, DBI and β -CCE prevented the stimulatory effect of diazepam on the binding of ^3H -GABA to crude synaptic membranes prepared from rat brain.

Proposed Course

Although DBI blocks the increase in diazepam binding induced by GABA, we cannot infer that DBI has a physiological role until we can show its synaptic location, its coexistence and release from GABAergic terminals and its action on the Cl^- channels that are regulated by GABA. In addition, the large molecular weight of DBI may suggest that we are isolating a precursor of a smaller molecular weight peptide, this could be the natural compound released

from nerve (GABAergic?) terminals. The rapid degradation of this small molecular weight peptide could have impaired its detection with present techniques.

Conclusions

Our data suggest that BDZ recognition sites are functionally associated with GABA receptors and that an endogenous brain peptide can modulate their function acting at the benzodiazepine recognition site as a naturally occurring anxiogenic compound.

The importance of these observations may go beyond the immediate explanation of the mode of action of BDZs and may represent the beginning of a better understanding of the role of GABAergic neurons in anxiety and in other mental disorders. In addition, these observations may help in elucidating details of the supramolecular nature of postsynaptic GABA and BDZ receptors and also may offer a simple means for studying the nature of endogenous materials involved in anxiety.

Publication:

Guidotti, A., Forchetti, C.M., Ebstein, B., and Costa, E.: Purification and characterization of an endogenous peptide putative effector for the benzodiazepine recognition site. In Proc. Symp. Pharmacology of Benzodiazepines, NIH, April 1982.

Project Description:

Glutamate

The objective of the study was to investigate the pharmacological regulation of the glutamatergic pathways in the rat brain.

The achievement of this objective required the development of a method allowing the measurement of glutamate turnover rate, and the determination of possible compounds which may serve as precursors for the synthesis of the releasable pool of glutamate. The study of glutamate turnover rate was approached in several ways.

1. The use of labelled compounds, as possible precursors for glutamic acid synthesis was investigated. ^{13}C -labelled proline was used as one of possible precursors, and was injected intracerebroventricularly (1 mg/brain). A GC-MS method was developed for the measurement of proline, using a single-step PFIA-HFIP derivatization and monitors ion fragments: $m/e = 216$ for proline and 220 for ^{13}C -proline. The labeled proline was found to be incorporated into the rat striatum with the maximal specific activity at 10 min after injection, however, the incorporation of ^{13}C into glutamic acid was not observed. The investigation of possible glutamate precursors will be pursued using in vitro techniques.
2. The kinetic of labelled glutamate incorporation into the glutamate pool of various brain structures was studied. Deuterium-labeled L-glutamic acid was injected intracerebroventricularly (10 $\mu\text{moles/kg l.w.}$), and the kinetics of incorporation into cortex, striatum, hippocampus and septum was determined using GC-MS technique. In all brain parts the maximal concentration of labelled glutamate was reached within 5 min after injection and then gradually decreasing. In the septum the amount of the labelled glutamate constituted 15% while in the other studied tissues labelled glutamate was 5% of the total glutamate pool. Further investigation will determine the kinetics of the decay of the labelled glutamate under conditions of stimulation and lesions of possible glutamatergic pathways.

Several degenerative neurological disorders such as Huntington's chorea, Parkinson's disease and spinocerebellar ataxia are characterized by selective premature loss of nerve cells. In these disease states the decreased catabolism of glutamate at the nerve terminals could result in an increased amount of the neurotransmitter at the synapses, leading to over-excitation and neuronal degeneration. Thus, the measurement of glutamate turnover in the brain may contribute to the knowledge of the underlying disease mechanisms of several degenerative neurological disorders.

Studies in progress include the pharmacological regulation of glutamate synthesis and release from nerve terminals. In future studies the regulation of the releasable neuronal pool of glutamic acid by various neurotransmitters will be investigated.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01520-06 SMRP | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The dynamics of catecholamine metabolism in the CNS: Biochemical pharmacology of the spinal cord catecholaminergic neurons | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">F. Karoum</td> <td style="width: 20%;">Staff Fellow</td> <td style="width: 15%;">SMRA</td> <td style="width: 20%;">NIMH</td> </tr> <tr> <td>Other:</td> <td>N. H. Neff</td> <td>Chief</td> <td>SMRP-B</td> <td>NIMH</td> </tr> </table> | | | PI: | F. Karoum | Staff Fellow | SMRA | NIMH | Other: | N. H. Neff | Chief | SMRP-B | NIMH |
| PI: | F. Karoum | Staff Fellow | SMRA | NIMH | | | | | | | | |
| Other: | N. H. Neff | Chief | SMRP-B | NIMH | | | | | | | | |
| COOPERATING UNITS (if any) Adult Psychiatry Branch | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | | | | | | | | |
| SECTION Biochemical Pharmacology | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.2 | PROFESSIONAL: 0.2 | OTHER: 0 | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to identify and study <u>the metabolism of catecholamines in spinal cord</u> and to evaluate the factors that influence <u>amine metabolism</u> . Our present interest is to determine the relative rates of <u>dopamine</u> and <u>norepinephrine</u> formation in various regions of the spinal cord. | | | | | | | | | | | | |

Proposed Course:

This project has been terminated.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01521-07 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Functional role of substance P and other peptides in nervous system | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> PI: J. P. Schwartz Research Chemist SMRP NIMH </div> | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 0.2 | OTHER: 1.0 |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS </div> | | |
| SUMMARY OF WORK (200 words or less - underline keywords) In studies on the function of <u>substance P</u> and on the effect of drugs on its distribution, the possibility that a pool of <u>substance P precursor</u> exists must be considered. The <u>chick embryo dorsal root ganglion</u> contains a molecular species of high molecular weight immunoreactivity which could function as a precursor in the formation of substance P. The content of this possible precursor is regulated by treatment of ganglia with <u>nerve growth factor</u> . Substance P, and its apparent precursor, have also been found in superior cervical ganglia, probably located in interneurons, as well as in many other tissues. Studies are being carried out to examine the effect of exposure of animals to <u>anti-NGF antiserum</u> on the substance P content of these different tissues. | | |

Project Description:

Evidence is accumulating that many peptides, as well as protein hormones and transmitters, are synthesized as inactive precursors, and that the conversion of the precursor to the active component is an important step in the regulation of the modulatory peptide. We have used chick embryo dorsal root ganglia, which contain substance P cell bodies, to search for a precursor to substance P. Incubation of the ganglia in vitro for 20-24 hrs resulted in an increase in the concentration of substance P; this increase was blocked by cycloheximide. In addition, incubation of 9 day chick embryo ganglia with β -nerve growth factor resulted in a further increase in the substance P content of the ganglia, an increase also prevented by cycloheximide. Gel chromatographic analysis of extracts revealed the presence of 2 peaks of substance P-like immunoreactivity (SPLI), one of which eluted at the same position as authentic substance P; the size of the other peak, of a molecular weight larger than substance P, was increased following NGF treatment. We believe this peak may be a precursor to substance P.

In order to study the metabolism, as well as the development, of various peptidergic neurons, we have used animals exposed to antiserum against NGF or treated with capsaicin. These two treatments have certain effects in common. In animals exposed in utero or as newborns, there is a loss of substance P-containing cells from sensory ganglia, with a corresponding depletion of substance P in the spinal cord and skin. Preliminary results show the same sort of changes for somatostatin, another putative transmitter in sensory ganglia. In adult animals, in contrast, there is a depletion of the substance P content of these tissues with no loss of cell number. The effect of anti-NGF in the adult animals is surprising since sensory ganglia have been thought to lose their NGF responsiveness during embryological development. Studies with the anti-NGF treated animals have shown that substance P-containing neurons in adrenal medulla and ileum are also NGF-responsive, whereas those of the submaxillary gland, the retina, and a variety of brain regions are not. In addition to looking at other peptides, use of these animals allows us to examine interactions between comodulators and between neurons.

We plan to measure other peptides in order to determine how wide-spread the dependence on NGF is. In addition, we will use the animals to examine interactions between comodulators and between neurons. For example, although loss of substance P-terminals in the spinal cord had no significant effect on opiate binding, recent immunohistochemical results suggest that GABA binding may change. The potential role of peptides as neurotransmitters and/or neuromodulators in the nervous system has expanded our knowledge of how the brain functions but has also expanded the possible sites where defects or altered metabolism could result in mental disorders. It thus becomes imperative to learn as much as possible about this new class of neuroactive compounds.

Publications:

Ross, M., Lofstrandh, S., Gorin, P.D., Johnson, E.M., and Schwartz, J.P.: Use of an experimental autoimmune model to define nerve growth factor dependency of

peripheral and central substance P-containing neurons in the rat. J. Neurosci.
1: 1304-1311, 1981.

Schwartz, J.P., Pearson, J., and Johnson, E.M.: Effect of exposure to anti-NGF
on sensory neurons of adult rats and guinea pigs. Brain Res., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01524-07 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Evidence for peripheral dopaminergic neurons | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | Z. Lackovic M. Relja N. H. Neff | Visiting Associate Guest Worker Chief |
| | | SMRP SMRP SMRP-B |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Biochemical Pharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.4 | PROFESSIONAL: 0.4 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| We previously provided experimental evidence that many <u>peripheral tissues</u> contain rather high concentrations of <u>dopamine</u> suggesting that it may be a neurotransmitter in addition to being a <u>precursor</u> for norepinephrine. Our current objective is to provide evidence that there are specific receptors for dopamine in peripheral tissues. | | |

Project Description:

There is now experimental evidence for the presence of dopaminergic neurons (see Z01 MH 01524-06 SMRP) in peripheral organs. Our current object is to provide evidence that there are dopamine receptors in the organs where there is evidence for dopaminergic neurons.

Plasma membranes were prepared from the rat vas deferens, a structure which presumably contains dopaminergic neurons, and standard radioligand binding techniques applied to evaluate the presence of dopamine receptors.

Specific dopaminergic receptors were identified in membranes prepared from rat vas deferens with the ligand ^3H -haloperidol. Binding was saturable and a Scatchard analysis of the data revealed a $K_d = 21 \text{ nM}$ and a $B_{\text{max}} = 74 \text{ fmol/mg prot.}$ Dopamine displaced 50% of ^3H -haloperidol binding at a concentration of about $10 \text{ }\mu\text{M}$, while norepinephrine, epinephrine and serotonin were practically ineffective at this concentration. Our results support the notion that there are specific dopaminergic receptors in the rat vas deferens. We speculate that some of the known effects of dopamine and dopaminergic drugs on sexual behavior may be mediated peripherally and not solely via the CNS as is usually assumed.

Schizophrenia and Parkinsons disease are associated with abnormal metabolism of dopamine. Some of the symptoms of these diseases may be related to a deficiency of peripheral dopaminergic neurons. Moreover, some of the side effects of drugs that act on the central dopaminergic system may be the consequences of actions on peripheral dopaminergic neurons or their receptors. Our studies are an attempt to answer some of these important questions.

In the future we will direct our interest toward establishing the possible physiological role of peripheral dopamine.

Publication:

Lackovic, Z., Relja, M., and Neff, N.H.: Catabolism of endogenous dopamine in peripheral tissues: Is there an independent role for dopamine in peripheral neurotransmission? J. Neurochem. 38: 1453-1458, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01525-06 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Regulation of gene expression and protein synthesis of nervous system- derived tissues | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | J. P. Schwartz E. Costa P. Onali F. Tang | Research Chemist Chief Visiting Fellow Guest Worker |
| | | SMRP SMRP SMRP SMRP |
| | | NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 2.2 | PROFESSIONAL: 2.2 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Nerve growth factor</u> (NGF) is synthesized and secreted by rat <u>C6 glioma</u>, a nervous system-derived cell line. The NGF content of these cells is increased by β-adrenergic agonists, which cause an elevation of cyclic AMP content and the concomitant activation of cAMP-dependent protein kinase. The increase of NGF content does not depend on the increase in protein synthesis which is elicited by isoproterenol, whereas the increase of <u>cyclic nucleotide phosphodiesterase</u> (PDE) does. There are two forms of PDE in the cells; the cyclic AMP-specific form is induced by β-adrenergic agonists, whereas the second form hydrolyzes cAMP or cGMP and is regulated by calcium and <u>calmodulin</u>. The induction of PDE requires translocation of the catalytic subunit of cytosol protein kinase into the nucleus, a process which is inhibited by treatment of the cells with vinblastine or colchicine. Phosphorylation of acidic nuclear proteins is also required for PDE induction; this phosphorylation can be blocked by cordycepin. RNA polymerase II is required for synthesis of PDE mRNA and treatment of the cells with either actinomycin D or α-amanitin inhibits RNA polymerase and blocks PDE induction. </p> | | |

Project Description:

The nervous system-derived cell line C6 glioma contains a β -adrenergic receptor through which cyclic AMP-dependent functions in the cell can be regulated. Among the consequence of isoproterenol activation of adenylate cyclase in the C6 glioma cells are an induction of cyclic AMP phosphodiesterase (PDE) and an increase in nerve growth factor content. The increase of PDE is a process which reaches a peak in 3-4 hrs and requires new protein synthesis. The cell cytoplasm contains 2 forms of PDE, which are separable on a DEAE-Sephacel column. The first form utilizes both cyclic AMP and cyclic GMP as substrates and is activated by calcium and calmodulin. The second form, which acts only on cyclic AMP, is specifically induced by isoproterenol treatment. This form is not affected by either calcium plus calmodulin or cyclic GMP (up to 100 μ M). Further purification revealed a single peak of activity with an apparent MW of 54,000, whose specific activity is increased following β -adrenergic stimulation. Kinetic analysis revealed a non-linear Hofstee plot with apparent K_m values of 2-5 μ M for cyclic AMP.

Characterization of protein kinase activation is the first step in determining how the cyclic AMP-activated protein kinase is increasing the β -NGF and PDE content of the cells. Following activation of the cyclic AMP-dependent protein kinase, there is a translocation of the catalytic subunit of the kinase from the cytosol to the nucleus. Pretreatment of glioma cells with vinblastine or colchicine blocks the increase of nuclear protein kinase and the increase of PDE activity elicited by isoproterenol. These results suggest first that the translocation of activated subunits of protein kinase from cytosol to the nucleus is required for the induction of new synthesis of PDE molecules. In addition, since vinblastine and colchicine inhibit microtubule polymerization, ~~such~~ results suggest that microtubules are involved in the translocation process. Regulation of protein phosphorylation depends on activation of protein kinase, location of the activated enzyme, and specific substrates present in the sites where activated enzyme is located. An as yet unidentified nuclear acidic protein(s) represents a substrate for the translocated kinase. At 1-2 hrs following isoproterenol, there is increased phosphorylation of the acidic protein fraction, with no change in the degree of phosphorylation of either histones or the remainder of the nuclear proteins. Both the increased phosphorylation of acidic proteins and the PDE induction can be blocked by cordycepin, suggesting that acidic proteins regulate expression of the gene for PDE. RNA polymerase II is also required for induction of PDE. Its activity in vivo and in vitro as well as the induction of PDE can be blocked by either actinomycin D or α -amanitin.

Since neither cycloheximide nor vinblastine affect the increase in β -NGF content elicited by isoproterenol, it is inferred that at least the early (3 to 6 hr after isoproterenol) increase in NGF is not the result of new protein synthesis. We believe that this increase is the result of the activation of a process whereby pro- β -NGF is converted into NGF. We are currently in the process of isolating the gene for NGF and propose to use it to determine whether cyclic AMP regulates expression of the mRNA for NGF in C6 cells, and how the precursor pro-NGF is biosynthetically regulated.

In parallel with these studies, we have undertaken a series of experiments using a cDNA coding for proenkephalin as a probe in order to determine whether different tissues contain multiple genes and/or multiple mRNAs encoding proenkephalin. We propose to study whether pharmacological or physiological manipulations can alter the level of expression of the mRNA(s). Primary cultures of bovine adrenal chromaffin cells, which are responsive to both acetylcholine and certain opiates, will be used for the initial experiments. We will then examine the effect of reserpine on these cultures' content of proenkephalin mRNA. Chronic treatment of animals with haloperidol affects long-term opiate peptide content of rat striatum. We intend to determine whether this regulation occurs at the level of mRNA.

Both neurotransmitters and drugs have certain long-term effects or effects which appear only after chronic exposure. Some of these effects occur at the level of transcription and the technique of molecular biology utilizing specific gene probes will enable a better understanding of changes occurring in the brain as a result of either chronic drug treatments or of disease-induced changes in transmitter release or function. Such an understanding will enable us to design better drugs or treatments for mental diseases.

Publications:

Onali, P., Schwartz, J.P., Hanbauer, I., and Costa, E.: Regulation by a β -adrenergic receptor of a Ca^{++} -independent adenosine 3'-5' cyclic monophosphate phosphodiesterase in C6 glioma cells. Biochem. Biophys. Acta 675: 285-292, 1981.

Schwartz, J.P.: RNA polymerase II in C6 glioma cells: α -Amanitin blockade of cyclic AMP phosphodiesterase induction by β -adrenergic stimulation. Exp. Cell Res. 137: 39-45, 1982.

Schwartz, J.P., and Onali, P.: Regulation of intracellular and nuclear metabolism by stimulation of β -adrenergic receptors on C6 glioma cells. In Usdin, E., Weiner, N. and Youdim, M. (Eds.): Function and Regulation of Monoamine Enzymes - Basic and Clinical Aspects, Macmillan, 1982.

Schwartz, J.P.: Cyclic AMP-mediated modulation of gene expression. In Hanin, I. (Ed.): Dynamics of Neurotransmitter Function, New York, Raven Press, in press.

Schwartz, J.P. and Onali, P.L.: Beta-adrenergic receptor regulation of a cyclic AMP phosphodiesterase in C6 glioma cells. Adv. Cyclic Nucl. Res. 15: in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01526-06 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Modulation of tyrosine hydroxylase activity in retina | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | J. Cohen M. Economou- Hadjiconstantinou N. H. Neff | Staff Fellow Guest Worker Chief |
| | | SMRP SMRP SMRP-B |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) Dr. M.P. Iuvone, Dept. Pharmacology, Emory U. School of Medicine, Atlanta, Ga. | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Biochemical Pharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.4 | PROFESSIONAL: 1.4 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Our present interests are three-fold: 1) to determine whether <u>tyrosine hydroxylase</u> of <u>dopamine-containing amacrine cells</u> becomes tolerant to activation by neuroleptic drugs and to evaluate whether tolerance to <u>neuroleptic drugs</u> alters activation by a <u>photic stimulus</u> ; 2) to characterize and compare the activation of retinal tyrosine hydroxylase induced <u>in vivo</u> by photic stimulation and <u>in vitro</u> by <u>cAMP-dependent protein kinase</u> ; and 3) to investigate some of the properties of the <u>epinephrine</u> -containing system of retina. | | |

Project Description:

Dopamine, a putative neurotransmitter in the central nervous system of mammals, is contained in a subpopulation of retinal amacrine neurons. Exposure to light activates retinal tyrosine hydroxylase activity, concomitant with an increase of dopamine synthesis (see Z01 MH 01526-02,03). Activation of the enzyme is characterized by a decrease of the K_m for the pteridine cofactor. Neuroleptic drug treatment results in activation of retinal tyrosine hydroxylase activity with a decrease of the K_m for the pteridine cofactor. In vitro tyrosine hydroxylase can be activated if incubated under protein phosphorylating conditions in the presence of cAMP. Our first objective was to determine whether chronic treatment with neuroleptic drugs might induce permanent changes of the retinal enzyme and thus alter the response to light. In a second series of studies we compared the characteristics of the enzyme after activation in vivo by light and activation induced in vivo by cAMP dependent protein kinase. And finally, we established and evaluated the response of an epinephrine containing system of the retina to light and pharmacological agents.

- I. Animals were placed in a light controlled environment and chronically treated with haloperidol. Enzyme activity was analyzed by standard procedures. Haloperidol's ability to activate tyrosine hydroxylase was diminished 24 hours after terminating 22-30 days of treatment with haloperidol. The retinal enzyme system was also tolerant to activation by other neuroleptic drugs. In contrast, exposure of the animals to light activated the enzyme to the same extent in chronic haloperidol treated and control animals. Apparently activation of retinal tyrosine hydroxylase by haloperidol and light occurs by independent mechanisms.
- II. There were similarities between activation of tyrosine hydroxylase in vivo by light and in vitro by protein phosphorylation. Both treatments decreased the apparent K_m of the enzyme for the pteridine cofactor. Both treatments also resulted in similar shifts of enzyme activity when studied at different values of pH. When retinal extracts containing tyrosine hydroxylase that were activated either in vivo by photic stimulation or in vitro by protein phosphorylation were incubated at 25°, the enzyme was inactivated in a similar time-dependent manner. The inactivation of the enzyme following both procedures for activation was prevented by the presence of high concentrations of sodium pyrophosphate, an inhibitor of phosphoprotein phosphatase. Moreover, activation by photic stimulation was not addition to the activation in vitro by protein phosphorylation. These findings suggest that the activation of retinal tyrosine hydroxylase in vivo may be mediated by phosphorylation of the enzyme or some effector molecule associated with the enzyme.
- III. While investigating dopamine metabolism in rat retina we identified both norepinephrine and epinephrine. Moreover, retinal homogenates were capable of methylating phenylethanolamine using S-adenosylmethionine-³H as the methyl donor. The phenylethanolamine N-methyltransferase inhibitor SKF 64139 blocked enzyme activity and there was a concomitant rise of norepinephrine and a fall of epinephrine content of retina. There were no significant changes of enzyme activity in rats killed in the dark when

compared with light. In contrast with enzyme activity, there was a significant rise of norepinephrine and a significant fall of epinephrine content in animals killed in the dark. Apparently light and dark influence the norepinephrine and epinephrine content of retina but not phenylethanolamine N-methyltransferase activity. This finding suggests that endogenous S-adenosylmethionine may be rate-limiting for epinephrine formation in retina.

Many of the drugs that are used to treat human mental disorders modify catecholaminergic neuronal function. Our studies are providing the bases for understanding the pharmacology of these drugs as well as the side effects associated with therapy.

Our future efforts will be directed towards evaluating the effects of neuroleptic drugs therapy to pregnant female rats on their offspring. There is now evidence that growth and development of brain can be influenced by drugs in utero.

Publications:

Cohen, J., and Neff, N.H.: Activation of retinal tyrosine hydroxylase: Tolerance induced by chronic treatment with haloperidol does not modify the response to light. J. Pharmacol. Exp. Ther., in press.

Iuvone, P.M., Rauch, A.L., Marshburn, P.B., Glass, D.B., and Neff, N.H.: Activation of retinal tyrosine hydroxylase in vitro by cyclic AMP-dependent protein kinase: Characterization and comparison to activation in vivo by photic stimulation. J. Neurochem., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01527-06 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Studies of endogenous opioids using HPLC | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. L. Meek Pharmacologist SMRP NIMH | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Group on High Pressure Liquid Chromatography | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.9 | PROFESSIONAL: 0.9 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>HPLC</u> is the most powerful technique available for <u>peptide separation</u> . However, the usefulness of HPLC for <u>peptide measurement</u> has been limited by the poor sensitivity of detection with UV monitors operating at 210 nm. Twenty compounds were tested for ability to rapidly form "clean" derivatives of peptides which could be detected with high sensitivity by absorbance or electrochemical detectors. Two compounds were synthesized which react rapidly ($T_{1/2}$ less than 1 min) with amino groups of peptides forming hydrophilic derivatives with a 50-500 fold improvement in detection limits over conventional methods. An acylating agent, 2 carboxy, 4,6-dinitrofluorobenzene (CDNFB) allows detection of 10 pmol of a dipeptide by absorbance at 360nm in comparison to the 500 pmol limit of the underivatized dipeptide at 210nm. An acylating agent, 3,6-dinitro phthalic anhydride forms a hydrophilic peptide derivative which can be detected electrochemically by reduction at -0.3 V at the 1 pmol level. | | |

Project Description:

High pressure liquid chromatography (HPLC) is the most powerful technique currently available for separation of small peptides. The usefulness of the technique for specific measurement of peptides has been limited by poor sensitivity of detection (0.2-2 nmol) possible with absorbance measurement at 210 nm of underivatized peptides. Development of more sensitive techniques by which peptides can be specifically detected would be a major advance in studies of neuropeptide identification, regional distribution, co-localization etc. In this project, a large number of potential derivatizing agents were examined with regards to suitability as peptide reagents: low lipophilicity, high molar absorbance in the visible region, ease of reduction/oxidation on glassy carbon electrodes, purity and stability of reagent and derivatives, and rates of reaction with various functional groups on peptides. Several types of reactions for the amino group were examined. These included: acylating agents (acid anhydrides and active esters), arylating agents ("activated" halobenzenes), reductive amination (Pyridoxal phosphate+ NaBH₄) and oxidative amination. Derivatives were prepared of simple peptides (e.g. val-val, met-enkephalin) and simple model compounds with possible interfering functional groups (e.g. N-acetyl tyrosine and N-acetyl cysteine). The compounds were separated by HPLC, retention times, detection limits, spectra and electrochemical characteristics and rates of reaction were examined.

MAJOR FINDINGS

- 1) Reagents were found which can a) be easily prepared in pure form, b) react rapidly with amino groups on peptides giving single derivatives) c) improve sensitivity of detection by 50 to 500 times, d) do not add excess lipophilicity to the peptide (and thereby make separation more difficult).
- 2) The reagents are 2 carboxy, 4,6 dinitro fluorobenzene (CDNFB), an analog of Sangers's reagent (dinitrofluorobenzene) which is more reactive, and much more hydrophilic, and 3,6 dinitrophthalic anhydride (DNPT).
- 3) CDNFB can be prepared by nitration of fluorobenzoic acid with sulfuric acid/sodium nitrate.
- 4) CDNFB improves detection limits for val-val from 500 pmol for the underivatized compound (absorbance at 210 nm) to 10 pmol for the derivative (360 nm).
- 5) CDNFB reacts very rapidly with N-terminal amino groups of peptides (at pH 9, T_{1/2} = .75 min), slower with side chain (lysine) amino groups (T_{1/2} = 5.5 min) and still slower with phenolic groups of tyrosine (T_{1/2} = 115 min).
- 6) DNPT can be prepared from its commercially available salt by extraction from acid and dehydration with acetic anhydride.
- 7) the limit of detection of DNPT val-val using reductive electrochemistry is about 1 pmol, i.e. 10 fold lower than detection of the CDNFB derivative.
- 8) DNPT reacts extremely rapidly with N-terminal amino groups (at pH 8, T_{1/2} is less than 10 sec). Reaction with phenolic and imidazole groups was not detectable.

SIGNIFICANCE

There is an acute need to be able to measure changes in the concentration of peptides in brain as affected by physical and pharmacological variables without the specificity problems inherent in immunoassays. The compounds described here (or similar ones for other peptide functional groups) should make possible the direct estimation of peptides in tissue.

FUTURE COURSE

The future course will take two directions: 1) using the derivitizing agents prepared in this project, specific techniques will be developed for a wide range of important peptides, e.g. TRH, cholecystokinin, dynorphin, peptide Y etc.; 2) general peptide methods will be developed analogous to "amino acid analysis" for amino acids which will examine the content of all the peptides of a given size/polarity/charge. Such a technique will make it possible to search for new transmitter/modulator peptides. "Peptide analysis" will be performed in brain nuclei to look for pathway-specific compounds.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01531-05 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Nerve growth factor in human fibroblasts from controls and familial dysautonomia patients | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | J. P. Schwartz J. Byrd | Research Chemist Guest Worker |
| | | SMRP SMRP NIMH NIMH |
| COOPERATING UNITS (if any) Department of Human Genetics, Yale University Medical School, New Haven, Connecticut | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.5 | PROFESSIONAL: 1.5 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Skin <u>fibroblasts</u> from human controls or from patients with <u>familial dysautono-</u> <u>mia</u> (FD) have been grown in tissue culture. <u>β-Nerve growth factor</u> (NGF) has been measured in extracts from both type of cells, using a radioimmunoassay and a bioassay. The FD cells have contents of NGF as measured by RIA comparable to the controls. However, the biological activity of the FD samples is about one- tenth that of the controls when expressed as biological units of activity per ng β-NGF as measured in the RIA. Stimulation of β-adrenergic receptors in both cell lines with l-isoproterenol results in a 17-170 fold rise in intracellular cyclic AMP in 10 min. The NGF content of the control fibroblasts increased 50- 300% after 3-4 hrs of exposure to isoproterenol whereas there was no change in the NGF content of the FD cells at any time. The NGF of heterozygote parents is comparable to that of controls, whereas the NGF of amniotic fluid fibroblasts has a lower activity. Assays are being carried out on fibroblasts from women at risk for FD, to determine the feasibility of prenatal diagnosis. | | |

Project Description:

Familial dysautonomia (Riley-Day Syndrome, FD) is an autosomal recessive disease, in which patients have impaired autonomic and sensory functions. Symptomatically, these patients resemble animals that have been treated with antiserum to NGF. It has been suggested that there may be an impairment of NGF associated with the disease, and there are reports that FD patients have elevated serum NGF levels.

We have chosen to use skin fibroblasts from FD patients, and fibroblasts from paired controls, to study this problem further. The fibroblasts are grown in monolayer culture, using early passage cells and standard tissue culture procedures. NGF is assayed using a radioimmunoassay which detects β -NGF as well as the β -component of 7S NGF, and also a bioassay in which neurite sprouting from 9-day chick embryo dorsal root ganglia is measured.

NGF remains essentially constant in both control and FD fibroblasts as they go from log to stationary phase growth. Control and FD cells have been found to contain comparable amounts of NGF as measured by radioimmunoassay. However, estimation of the NGF content of the same extracts by a bioassay procedure shows that the extracts from FD cells are much less biologically active than cells from paired controls. When expressed as biological units of NGF per ng β -NGF (as measured in RIA), there is a ten-fold difference in biological activity. In contrast, fibroblasts from patients with dystonia musculorum deformans, another genetically inherited neurological disease, contained levels of biological activity per ng of immunoreactive β -NGF that are similar to those of control extracts. Three dystonia lines, ten FD lines and ten control lines have been assayed. Comparable assays of cells from heterozygote parents and their FD children have demonstrated that the parents' cells resemble those of normal controls rather than an intermediate form. Assay of parental cells therefore cannot be used for diagnostic purposes at this point. Because of the increasing use of amniocentesis for prenatal diagnosis, we have been studying the feasibility of growing fibroblasts from amniotic fluid and measuring the NGF content by both radioimmunoassay and bioassay. We have now established a baseline NGF content for normal amniotic fluid cells (~ 0.2 B.U./ng of β -NGF) and have assayed cells from four pregnant women at risk; three of the women had normal children in agreement with our assay results. A large number of samples will be required to establish a correlation between the NGF activity of amniotic fluid cells and the presence or absence of familial dysautonomia.

Chemical characterization of the NGF present in the fibroblasts has proven to be extremely difficult because the NGF represents only 0.001% of the total cell protein. We have therefore chosen to use the techniques of molecular biology to advance this problem further. Messenger RNA which codes for pro-NGF has been isolated and size-purified from male mouse submaxillary glands. Injection of this mRNA into *Xenopus* oocytes results in the synthesis of pro- β -NGF which is detectable by RIA and bioassay following treatment with the converting enzyme γ . This mRNA has been used to prepare cDNA copies, inserted into a pBR plasmid, and clones of these plasmids are now being screened for the presence of the gene for β -NGF. Isolation of the NGF cDNA probe will allow us

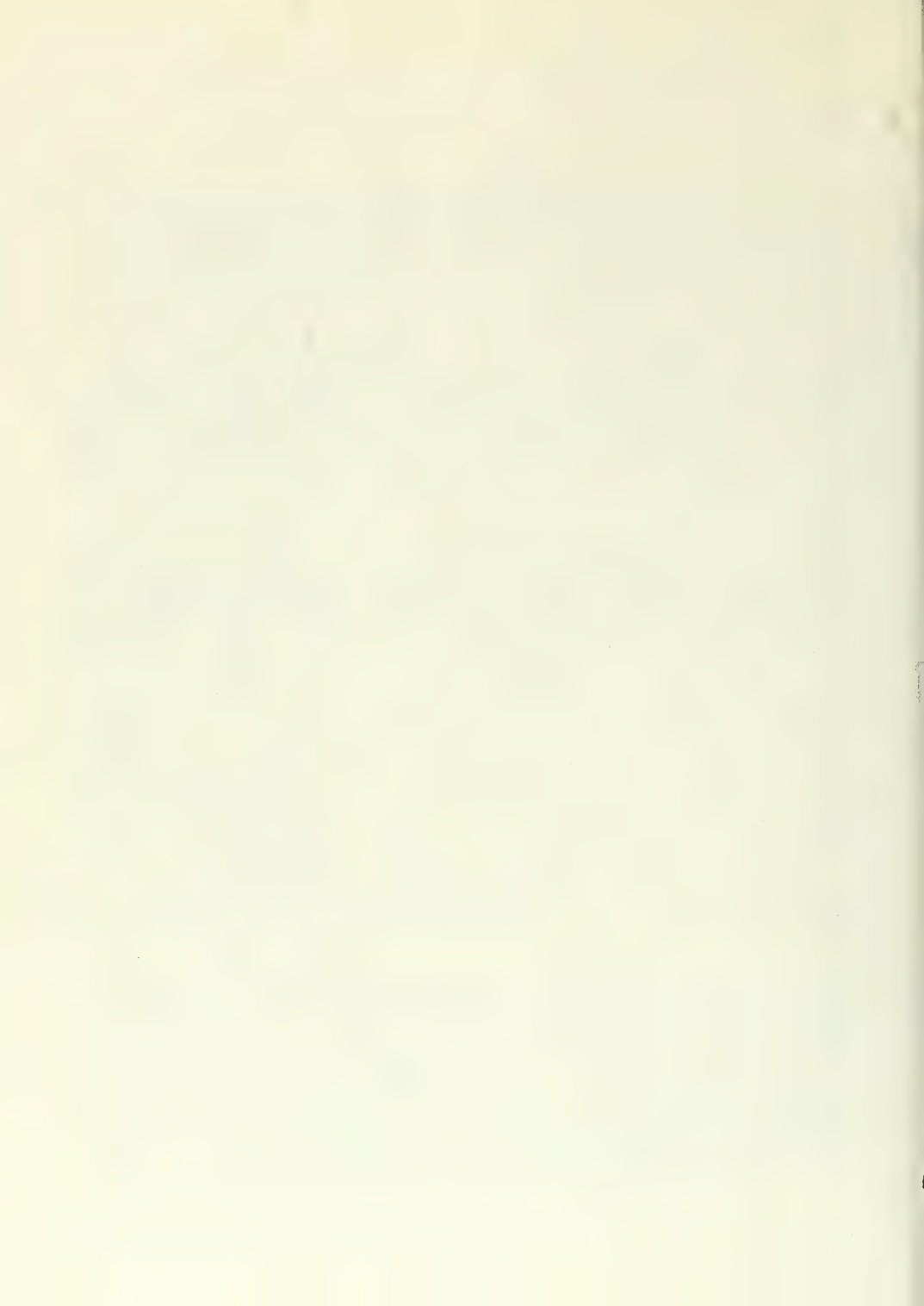
to examine directly the structures of the genes for human NGF in both dysautonomic and control fibroblasts.

In order to understand the biological effects of NGF better, studies have been initiated using the PC12 pheochromocytoma cell line which has NGF receptors and responds to NGF biochemically and biologically. These studies are centered on two major questions: 1) is the internalization of NGF along with its receptor required for its biochemical effects; 2) does NGF stimulate a tyrosine protein kinase associated with its receptor in the cell membrane, as has been demonstrated for other peptide hormones such as EGF and insulin.

Recent evidence suggests that many "nerve growth factors" exist, specific for different populations of neurons in either the CNS or PNS. A defect or loss of one of these factors would result in a disease of the nervous system. Using the gene probe for NGF under relaxed conditions may allow us to identify other "NGF"s and examine their role in mental health. Understanding how NGF exerts its physiological effects will provide clues as to how the other growth factors function.

Publication:

Breakefield, X.O., Edelstein, S.B., Grossman, M.H., and Schwartz, J.P.: Variations in MAO and NGF in cultured human skin fibroblasts. In Gershon, G.S., Matthysse, S., Cianarello, R.D. and Breakefield, X.O. (Eds.): Genetic Strategies in Psychobiology and Psychiatry. Calif., Boxwood Press, 1981, pp. 129-142.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01532-05 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Regulation of catecholamine receptor | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D. M. Chuang Chemist SMRP NIMH | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.6 | PROFESSIONAL: 0.6 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) In <u>frog erythrocytes</u> , the loss of membrane-bound <u>β-adrenergic receptor sites</u> during <u>isoproterenol-induced desensitization</u> is due at least in part to an <u>internalization</u> of the <u>β-receptor sites</u> . This receptor internalization is characterized by an increase in the number of soluble <u>β-receptor binding sites</u> . Experiments using various <u>lysosomotropic drugs</u> suggest that these soluble receptor sites are released from the endocytic vesicles into cytoplasm due to partial hydrolysis by lysosomal enzymes. Moreover, subcellular fractionation of erythrocytes by <u>Percoll</u> gradient centrifugation revealed that at least one species of <u>lysosomes</u> retains <u>β-receptors</u> and this receptor retention in lysosomes is enhanced when cells become desensitized. Treatments of cells with various inhibitors of <u>calmodulin</u> , a calcium binding protein, caused a time- and dose-dependent reduction in the extent of <u>β-receptor internalization</u> and down-regulation. These data suggest that calmodulin is involved in regulating the clustering of <u>β-receptors</u> in coated pits or vesicles which may be catalyzed by <u>transglutaminase</u> . Evidence is also presented that internalized <u>β-receptor sites</u> are recycled to the plasma membrane to restore the surface receptor density and functional sensitivity that are attenuated during desensitization. | | |

Project Description:

Receptors for neurotransmitters continuously adapt the efficiency of their operation to inherent conditions; the two extremes of this adaptation are termed "sub" and "super" sensitivities. This adaptation is viewed as an important factor of synaptic plasticity in mental health and disease. Hence, the practical importance of reaching a better understanding of the molecular mechanisms mediating receptor modulation is evident. The system of isolated frog erythrocytes has been used extensively as a model to study the mechanism of the desensitization of β -adrenergic receptors induced by a persistent receptor stimulation. This receptor desensitization is associated with a reduction in the density of plasma membrane-bound β -adrenergic receptor binding sites, suggesting that the density of cell surface receptors may control the magnitude of the receptor response. Using frog erythrocytes as a model system, we have obtained evidence indicating that receptor internalization is a mechanism for the loss of membrane-bound β -adrenergic receptor sites during isoproterenol-induced down-regulation of β -adrenergic receptors. This receptor internalization is reflected by an increase in the cytosolic fraction of the number of binding sites for [3 H]-dihydroalprenol, a β -receptor ligand. Numerous biochemical studies have demonstrated that this increase in the soluble β -receptor sites is tightly associated with the down-regulation of β -adrenergic receptors, that is the loss of membrane-bound β -receptor sites and the decreased responsiveness of the adenylate cyclase to β -receptor stimulation. In light of the findings that in many systems internalized receptor sites are delivered by some vesicular structures such as receptosomes to lysosomes where the ligand-receptor complexes are partially degraded, we have attempted to investigate whether soluble β -receptor binding sites detected during desensitization result from the processing of internalized receptors by lysosomal enzymes. Various lysosomal enzymes (acid hydrolases) have been detected in the homogenate of frog erythrocytes. Pretreatment of intact cells with various inhibitors of lysosomal enzymes caused a dose- and time-dependent reduction in the number of soluble β -receptor binding sites in isoproterenol-treated cells, whereas the level of membrane-bound receptors in these cells was unaffected. These results suggest that lysosomotropic drugs do not affect the down-regulation or internalization of β -adrenergic receptors but do inhibit the processing of the internalized β -receptor binding sites within the lysosome. The conclusion is further supported by an experiment using Percoll gradient to fractionate subcellular organelles in frog erythrocytes. One species of lysosomes (density: 1.15 g/ml), which appears to be free of plasma membrane, contained a fraction of membrane-bound β -adrenergic receptor binding sites. This fraction of membrane-bound receptors was markedly increased when desensitized cells were pretreated with chloroquine, one of the inhibitors of lysosomal enzymes. This accumulation of β -receptor sites in lysosomes in chloroquine-treated desensitized cells was associated with a reduction in the soluble binding sites detected near the top of the Percoll gradient.

Our previous investigation has demonstrated that exposure of frog erythrocytes to various inhibitors of transglutaminase can attenuate the isoproterenol-induced down-regulation and internalization of β -adrenergic receptors. Moreover there is a good correlation between the ability of these compounds to inhibit transglutaminase and their potency to block internalization. These results suggest that transglutaminase may mediate the clustering of ligand-

receptor complexes on clathrin-coated pits; this event is a prerequisite of the ligand-induced receptor internalization in various systems. Since transglutaminase is a calcium dependent enzyme capable of cross-linking proteins, I have investigated whether the apparent transglutaminase-mediated event involves calmodulin, a calcium binding protein. Indeed pretreatment of erythrocytes with various inhibitors of calmodulin (trifluoperazine, pimozide, chlorpromazine, mepacrine and tetracaine) caused a time and dose-dependent reduction in the extent of β -receptor internalization and down-regulation elicited by isoproterenol. Additional experimental results indicate that these drug effects are not due to an inhibition of the activity of phospholipase A_2 . These results are in line with the involvement of calmodulin in regulating the clustering of β -receptors in coated pits or vesicles. This possibility is strengthened by the recent finding that calmodulin is a component of coated vesicles and binds to coated vesicles with high affinity.

Based on the present study a model is proposed for the sequences of events that occur during β -receptor internalization in frog erythrocytes. A clustering of β -receptors may be triggered by the binding of a β -agonist and this receptor clustering on clathrin-coated pit may involve the enzyme transglutaminase. Eventually the coated pit pinches off the surface membrane to form an endocytic vesicle, which may be the receptosome as was reported previously. The receptosome then carries the β -receptor-agonist complexes to the lysosome where the receptosome may be partially degraded, while sparing the enclosed receptor in a soluble form present in the cytoplasm. Since the level of soluble β -receptors are returning to normal when β -receptor sites were restored during receptor resensitization and cycloheximide failed to block these events, it is proposed that internalized β -receptor binding sites can be recycled to the plasma membrane to restore the receptor sites and functional sensitivity that are lost during receptor subsensitivity. It should be stressed that this model is tentative and its final proof depends on morphological studies. Unfortunately morphological studies on β -receptor internalization has been hampered by the active passive diffusion in frog erythrocytes of isoproterenol and other known β -receptor-agonists (unpublished data). Thus the search for a labeled β -agonist which has high affinity for the receptor sites and is capable of inducing receptor internalization but is not taken up into erythrocytes nonspecifically would be the direction of future investigation. In summary, the present study has used frog erythrocytes as a model system to study the molecular mechanisms involved in the internalization and desensitization of β -adrenergic receptors. The information obtained may be helpful for future therapy of mental disorders resulting from malfunction of β -adrenergic receptors in the central or peripheral system.

Publications:

Chuang, D.M.: Inhibitors of transglutaminase prevent the agonist-mediated internalization and desensitization of β -adrenergic receptors. J. Biol. Chem. 256: 8291-8293, 1981.

Chuang, D.M.: Internalization of β -adrenergic receptor binding sites: Involvement of lysosomal enzymes. Biochem. Biophys. Res. Commun. 105: 1466-1472, 1982.

Chuang, D.M.: β -Adrenergic receptor internalization and processing: Role of transglutaminase and lysosomes. Molecular and Cellular Biochem., in press.

Chuang, D.M., Barbaccia, M.L., Brunello, N., and Kinnier, E.: Receptor regulation: An overview. In Hanin, I. (Ed.): Dynamics of Neurotransmission. New York, Raven Press, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01536-04 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Characterization of receptors for putative neurotransmitters | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | M. C. Olinas P. Onali N. H. Neff E. Costa | Visiting Associate Visiting Associate Chief Chief SMRP SMRP SMRP-B SMRP NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Biochemical Pharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 1.2 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to identify and study the <u>receptors</u> for <u>putative transmitter substances</u> . Our present objective is to: 1) determine if <u>muscarinic receptors</u> of <u>striatum</u> are coupled to <u>adenylate cyclase</u> and; 2) determine whether <u>muscarinic</u> and <u>dopaminergic receptors</u> coregulate striatal <u>adenylate cyclase</u> activity. | | |

Project Description:

Abnormal receptor function may play a role in the etiology of some forms of mental and neurological diseases. Our objective was to determine if muscarinic receptors of the striatum are coupled to adenylate cyclase and whether dopamine and muscarinic receptors are interactive.

Plasma membranes were prepared from rat striatum and adenylate cyclase activity determined by standard biochemical procedures. Acetylcholine inhibited basal adenylate cyclase activity of striatum in a concentration-dependent manner. Maximal inhibition was about 40% of basal activity and there was a requirement for GTP. Cyclase inhibition only occurred in the presence of muscarinic agonists and inhibition was prevented by atropine or scopolamine. Intra-striatal injection of kainic acid to destroy neuronal cell bodies abolished the inhibitory action of acetylcholine. These studies suggest that muscarinic receptors are coupled to striatal adenylate cyclase in an inhibitory manner. In a separate set of studies we found that acetylcholine receptors and dopamine receptors coupled to adenylate cyclase were interactive. Acetylcholine diminished the ability of dopamine to activate striatal adenylate cyclase. Kinetic analysis revealed that acetylcholine decreased the maximal velocity of the dopamine cyclase system in a non-competitive manner. The down regulation of the dopamine receptor by acetylcholine appeared to be a specific interaction as acetylcholine did not diminish activation of adenosine-stimulated adenylate cyclase. These studies demonstrate that there is a functional interaction between dopamine and acetylcholine on the same plasma membrane.

Most drugs used to treat schizophrenia interact with dopamine receptors thus implicating dopaminergic neurons in the disease process. Our studies are providing basic information needed to understand normal physiological mechanism and how to control these mechanisms in disease. Future studies will be directed towards investigating the consequence of long-term treatment with drugs that occupy dopamine or muscarinic receptors on the dopamine-acetylcholine receptor interaction.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01537-04 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biochemical pharmacology of GABA receptor system | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | B. Wise D. Konkell A. Guidotti E. Costa | PRAT Fellow Chemist Chief Chief SMRP SMRP SMRP-N SMRP NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any): None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Neuroendocrinology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.8 | PROFESSIONAL: 0.3 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>GABA-modulin</u> , an endogenous membrane protein, which inhibits non competitively ³ H-GABA binding to synaptic plasma membranes, has been isolated and purified to homogeneity using acidic extraction followed by Sephadex column purification and HPLC. GABA-modulin is a peptide of 16,000 MW which inhibits the binding of ³ H-GABA and the GABA-induced stimulation of ³ H-diazepam binding to synaptic membranes. The GABA-modulin molecule contains an abundance of hydrophilic residues (especially basic residues), and no cystein or GABA. End group analyses of GABA-modulin indicated that the carboxy terminus is free and is histidine, while the N-terminus is blocked. GABA-modulin can be phosphorylated by a Ca ⁺⁺ -calmodulin dependent protein kinase. The role of GABA-modulin in the control of GABA receptor system function is presently being investigated. | | |

Project Description:

When GABA receptor sites are occupied by the endogenous agonist, the ion channel located in the membrane becomes permeable to Cl^- ion, resulting in a hyperpolarization or depolarization of the receptive neuron depending on the concentration of Cl^- in the surrounding medium. The goal of this project is to provide a better understanding of the function of the GABA receptor complex and in particular the coupling of GABA recognition site with the Cl^- channel as a first step in developing new potent and specific drugs which like benzodiazepines can facilitate the stimulation of GABA receptors.

Our studies indicate that freshly prepared crude synaptic membranes, purified synaptic plasma membranes, or membranes from neuroblastoma clonal cell lines, contain only one population of ^3H -GABA recognition sites. Repeated washings of these membranes combined with freezing, thawing and treatment with Triton X-100 unmasks an additional population of GABA recognition sites characterized by high affinity for the agonist. Subjecting the Scatchard plot of these binding studies to the graphic analysis of Rosenthal for one ligand and two types of binding sites, the total density of GABA receptor sites and the relative portion of high (K_d 20-40 nM) and low affinity component (K_d 200-400 nM) of ^3H -GABA binding can be estimated.

The first step of the extraction procedure employed was homogenization of the tissue in hot (80°) 1 N acetic acid. This method was preferred to extraction procedures at a neutral pH because acid extraction at 80° destroyed proteolytic activity. The extraction in hot acetic acid was followed by 60% ammonium sulfate precipitation, Sephadex G-100 and G-75 column chromatography and anion exchange chromatography. Final purification was achieved by applying the material to a reverse phase HPLC Bio-Sil ODS-10 column.

Using this technique, the material was purified to homogeneity in a single (30 min chromatographic run. The major peak of protein eluting from the reverse phase HPLC with 50% acetonitrile inhibited the high affinity (K_D 20 nM) ^3H -GABA binding (IC_{50} 0.5 μM). This activity, which was destroyed by hydrolysis with trypsin or chymotrypsin, was not due to contaminating GABA, as amino acid analysis of the material did not detect any GABA. HPLC, with different columns and buffer conditions, polyacrylamide gel electrophoresis at different pH, analysis of amino acid composition, and carboxyl terminal amino acid analysis concurred to support the view that GM was purified to homogeneity. The molecular weight of GM evaluated by 12% SDS gel (17,000) is in good agreement with the molecular weight calculated from the results of the amino acid composition (16,200).

Amino acid composition, anion exchange chromatography and acrylamide gel with urea at acidic pH revealed that the protein is basic in nature. In washed Triton X-100 or AgNO_3 -treated crude synaptic membranes, purified GM inhibited both binding of ^3H -GABA to the high affinity site and GABA induced stimulation of ^3H -diazepam binding with an IC_{50} of around 0.5 μM . This concentration was within the range of GM concentration present in brain. In fact, it can be calculated from the recovery studies with ^{125}I -GM that the rat brain contained approximately 6 μM of GM. The action of GM is specific for GABA binding, because at doses up to 5 times higher GM failed to influence other ^3H -ligand

binding. In addition, the specificity of GM action is confirmed by the lack of effect by other proteins (histone small rat basic protein, lysozyme) of similar molecular weight and/or charge on ^3H -GABA binding.

Purification of GM has opened new interesting research approaches for the studies of the regulation of GABAergic transmission. For example, we are presently studying if GM is the only brain peptide that inhibits ^3H -GABA binding. Although the activity of GM was destroyed by trypsin or chymotrypsin digestion, we could not exclude the possibility that the GM we had purified was the precursor of the endogenous modulator; in fact, we have not yet studied the activity of GM fragments produced by more limited proteolysis.

Another important question is whether GM facilitates or inhibits the biological activity of GABA released by nerve stimulation. Preliminary experiments show that when GM, which down regulates ^3H -GABA binding, is injected into the cerebral ventricles, it exacerbates the convulsions induced by isoniazid, suggesting that the increase in free GM decreases the action of GABA.

Finally, the question of the mechanism by which GM inhibits the binding of ^3H -GABA remains to be explored in detail. Because GM inhibits the high affinity GABA binding in an apparently noncompetitive fashion, it is proposed that the mechanism is primarily allosteric in nature. In this regard, the observation that GABA-modulin is a good substrate for phosphorylation is interesting. It is therefore possible to study whether Ca^{2+} or cGMP- or cAMP-dependent phosphorylation is operative in regulating the degree of GM control of GABA recognition site down regulation.

Evidence has been accumulated to suggest that abnormalities in GABAergic function may be operative in determining the symptoms of Huntington's disease, Parkinson, epilepsy and possibly schizophrenia. Thus the biochemical and pharmacological characterization of GABA receptors modulation may be a relevant approach to find compounds which are capable of modifying the GABAergic system in man.

We are now developing a simple method to measure GABA-modulin content in different biological samples and to understand whether drugs that interfere with the action of GABA-modulin may be useful in the control of the GABA receptor system in neurological and/or psychiatric disorders.

Publications:

Massotti, M., Mazzari, S., Schmid, R., Guidotti, A., and Costa, E.: Endogenous inhibitors of Na^+ independent ^3H -GABA binding to crude synaptic membranes. Neurochem. Res. 6: 551-566, 1981.

Massotti, M., Guidotti, A., and Costa, E.: Purification and characterization of benzodiazepine and gamma aminobutyric acid recognition sites and their endogenous modulators. J. Neuroscience 1: 409-418, 1981.

Guidotti, A., and Ebstein, B.: Role of GABA-benzodiazepine receptor complex in epilepsy. In Morselli, P.L., Lloyd, K.G., Loscher, W., Meldrum, B., and

Reynolds, E.H. (Eds.): Neurotransmitters, Seizures and Epilepsy. New York, Raven, 1981, pp. 85-92.

Ebstein, B., Guidotti, A., and Costa, E.: Solubilization and characterization of the single components of the GABA receptor complex. In Okada, Y., and Roberts, E. (Eds.): Problems in GABA Research from Brain to Bacteria. Amsterdam, Excerpta Medica, 1982, pp. 348-354.

Costa, E.: The supramolecular organization of receptors for gamma aminobutyric acid (GABA). In Biggio, G., Costa, E., Gessa, G.L., and Spano, P.F. (Eds.): Receptors as Supramolecular Entities. Oxford, England, Pergamon Press, 1982, in press.

Costa, E., Corda, M.C., Wise, B., Konkell, D., and Guidotti, A.: Benzodiazepine and GABA interactions: Role of GABA-modulin. Benzodiazepine Conference, NIH, 1982.

Guidotti, A., Konkell, D.R., Ebstein, B., Corda, M.G., Krutzsch, H., Meek, J.L., and Costa, E.: Isolation, characterization and purification to homogeneity of GABA-modulin from rat brain. Proc. Natl. Acad. Sci. USA, submitted.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01540-04 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Enkephalin like peptides of adrenal glands | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | S. Govoni I. Hanbauer H.-Y.T. Yang E. Costa | Guest Worker Pharmacologist Pharmacologist Chief |
| | | SMRP SMRP SMRP |
| | | NIMH NIH NIMH NIMH |
| COOPERATING UNITS (if any) Section on Biochemical Pharmacology, NHL&B, NIH, Bethesda, MD | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.5 | PROFESSIONAL: 0.5 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Secretion of enkephalin like peptides from adrenal glands</u> into circulation was studied in dogs with indwelling cannulae in adrenal vein. The stimulation of the splanchnic nerve induces a voltage dependent increase of met⁵-enkephalin immunoreactive peptide (MEIP) in plasma. The MEIP secreted was identified to be met⁵-enkephalin using Bio-Gel P-2 column chromatography followed by high pressure liquid chromatography. The effect of splanchnic nerve stimulation was antagonized by hexamethonium suggesting that the release of MEIP was mediated through activation of <u>nicotinic receptor</u>. This suggestion was further supported by the fact that <u>dimethylphenylpiperazinium</u> induced an increase of circulating MEIP. Morphine injection was found to induce a pronounced increase of plasma MEIP in intact dogs but not in splanchnic nerve transected dogs. This effect was blocked by naloxone and also by hexamethonium. The results suggest that the effect of morphine is centrally mediated. This study clearly indicates that enkephalin-like peptides in chromaffin cells of adrenal glands can be secreted into circulation. </p> | | |

Proposed Course:

This project has been terminated.

Publication:

Govoni, S., Hanbauer, I., Hexum, T.D., Yang, H.-Y.T., Kelly, G.D., and Costa, E.: In vivo characterization of the mechanism that secrete enkephalin-like peptides stored in dog adrenal medulla. Neuropharmacology 20: 639-645, 1981.

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|---|--|--|--|--|--|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 MH 01542-04 SMRP | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | |
| TITLE OF PROJECT (80 characters or less) Amino acid neurotransmitters and hippocampal functions | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| PI: A. Di Lauro | | Visiting Associate | | SMRP | |
| Other: J. L. Meek | | Pharmacologist | | SMRP | |
| | | | | NIMH | |
| | | | | NIMH | |
| COOPERATING UNITS (if any) None | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | |
| SECTION Group on High Pressure Liquid Chromatography | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | |
| TOTAL MANYEARS: 0 | | PROFESSIONAL: 0 | | OTHER: 0 | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Aspartate</u> is an important excitatory transmitter in the brain, as well as a protein component and metabolic intermediate. An <u>aspartate binding</u> method was developed for use in brain which allows study of aspartate receptors. Two binding sites were demonstrated, differing in their sodium dependence, Kd, inhibition by other amino acids, and in their regional distribution. The ability to measure neuronal binding sites (receptors) opens up the possibility of studying interactions with other neurotransmitters, cotransmitters and neuromodulators, as well as the possibility of exploring aspartate pathways in areas of brain where detection of changes in aspartate content would not be possible. | | | | | |

Project Description:

Study of aspartate as a neurotransmitter has been difficult since there are no methods for 1) the histochemical localization of aspartergic neurons, 2) specific measurement of aspartate turnover in the pools that are involved in neurotransmission, and 3) measurement of release without the use of the rather nonspecific radioactive aspartate uptake methods. A few areas of brain (e.g. hippocampus and cochlear nucleus) have sufficiently dense innervation that lesion experiments can demonstrate aspartate loss. However, binding methods need to be developed that will allow study of all brain areas. We have now succeeded in detecting aspartate receptors on rat brain membranes via measurement of specific high affinity binding of ^3H -aspartate.

Major Findings

- 1) Two kinds of aspartate receptors can be detected in brain differing in their sodium dependence.
- 2) The sodium independent form had a low K_d (200 nM), low B_{max} (6 pmol/mg) high specificity (not inhibited by D-aspartate or L-glutamate), and decreased in content after destruction of intrinsic neurons with kainic acid. This site is probably a neuronal aspartate receptor.
- 3) The sodium dependent form had a higher K_d (500 nM) and B_{max} (100 pmol/mg), low specificity (inhibited by D-aspartate and L-glutamate) and was unaffected by kainic acid lesions. This binding probably represents an uptake site.
- 4) The sodium dependent and independent sites differ in their regional distribution; the former is more concentrated in the cerebral cortex, and less in the cerebellum, while the reverse is true for the sodium independent form.

This project has been terminated.

Publication:

Di Lauro, A., Meek, J.L., and Costa, E.: Specific high affinity binding of aspartate to rat brain membrane. J. Neurochem. 38: 1261-1267, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01543-03 SMRP |
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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of neurotensin in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | | |
|--------|--------------|--------------------|------|------|
| PI: | S. M. Govoni | Visiting Associate | SMRP | NIMH |
| Other: | J. S. Hong | Staff Fellow | SMRP | NIMH |
| | H.-Y.T. Yang | Pharmacologist | SMRP | NIMH |
| | E. Costa | Chief | SMRP | NIMH |

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

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| TOTAL MANYEARS: 0.6 | PROFESSIONAL: 0.4 | OTHER: 0 |
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Similarities have been shown to exist between neurotensin (NT) and neuroleptics by other investigators. Intrinsic enkephalinergic neurons in striatum (ST) and in nucleus accumbens (NA) appear to be regulated transynaptically raising the question whether a similar regulation is operative for NT containing neurons which are also present in these areas. Previous results indicated that in rat haloperidol injection can increase the NT selectively in NA and ST. The effect is observed either after acute or repeated treatment. We have now studied the NT content of various brain areas after treatment with different antipsychotic drugs. The results indicate that all the neuroleptics tested (chlorpromazine 6.0 mg/kg; trifluoroperazine 2.0 mg/kg; pimozone 1.5 mg/kg; haloperidol 1 mg/kg) increase the content of NT in NA and ST. On the contrary, promazine (10 mg/kg) and prometazine (25 mg/kg) which are structurally related to phenothiazines but with weak or no neuroleptic activity failed to increase the NT content of NA and ST. This effect is specific for NA and ST and it is not observed in preoptic area hypothalamus, septum, amygdala although these areas are enriched in NT. The neuroleptics induced increase of NT in NA and ST suggest a modulation of NT metabolism through dopaminergic synapses.

Proposed Course:

This project has been terminated.

Proposed Course:

This project has been terminated.

Proposed Course:

This project has been terminated.

Publication:

Stine, S.M., Yang, H.Y., and Costa, E.: Evidence for ascending and descending intraspinal as well as primary sensory somatostatin projections in the rat spinal cord. J. Neurochem. 38: 1144-1150, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01546-03 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Stimulation and homologous desensitization of cGMP synthesis by opioid, H1 histaminergic and muscarinic cholinergic agonists in N4TG1 cells | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | G. Cwynn E. Costa | Guest Worker Chief |
| | | SMRP SMRP |
| | | NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.1 | PROFESSIONAL: 0.1 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The <u>neuroblastoma clone N4TG1</u> possesses specific recognition sites for the putative neuromodulators <u>histamine</u> , <u>acetylcholine</u> and <u>enkephalin</u> . These sites are positively coupled with <u>guanylate cyclase</u> : agonist stimulation in the presence of a phosphodiesterase inhibitor causes a dose-related increase in <u>cellular cyclic GMP (cGMP)</u> content. A marked <u>refractoriness</u> or <u>desensitiza-</u> <u>tion</u> of this response develops after short-term agonist exposure. In each case this desensitization is specific or homologous and is not mediated by a de- crease in either the binding affinity or number of recognition sites. Agonist- induced desensitization of cGMP formation does not, furthermore, appear to be mediated by alterations in the specific activity of either phosphodiesterase or guanylate cyclase. Rather, the marked increase in the activation constant for cGMP formation in desensitized cells indicates that a change in the coup- ling function(s) between recognition site occupancy and enzyme stimulation has occurred. We are currently examining the roles of membrane calcium channels and phospholipid turnover in desensitization of cGMP synthesis in N4TG1 cells. | | |

Proposed Course:

This project has been terminated.

Publication:

Gwynn, G., and Costa, E.: Opioids regulate cGMP formation in cloned neuroblastoma cells. Proc. Natl. Acad. Sci. USA 79: 690-694, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRANURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01548-03 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Afferent control of serotonergic pathways | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | C. M. Forchetti J. L. Meek | Guest Worker Pharmacologist |
| | | SMRP SMRP |
| | | NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Group on High Pressure Liquid Chromatography | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0 | PROFESSIONAL: 0 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The afferents to <u>serotonin</u> (5HT) containing cells of the median <u>raphe nucleus</u> are known to include <u>GABA</u> , <u>substance P</u> and <u>met enkephalin</u> . The effect of injecting these substances into the median raphe nucleus was studied on serotonin turnover. GABA had a tonic inhibitory effect on 5HT activity; substance P and met enkephalin both had an excitatory effect on 5HT activity. Since GABA interneurons might be involved in control of serotonergic activity, we also examined the effect of substance P on the turnover of GABA in the median raphe nucleus. As an estimation of GABA turnover, we measured the accumulation of GABA after inhibition of its catabolism with gabaculine. Stereotaxic injections of this drug in the median raphe caused an increase in GABA that was linear with time for up to 90 min, and rapid in onset. Substance P injection into the median raphe increased the rate of accumulation of GABA by 30%. The data suggest that the predominant effect of substance P is directly on 5HT cell bodies, and does not act via a GABA interneuron. | | |

Project Description:

We have previously found that GABA has a tonic inhibitory effect on the serotonin cells in the median raphe nucleus. We wished to extend our studies to two other transmitters known to occur in the raphe (substance P and met-enkephalin), and also to examine whether effects on serotonin turnover might be mediated by GABA interneurons. We studied 5HT and GABA turnover in the raphe after local injection of substance P and a metabolically stable met-enkephalin analog. To estimate GABA turnover, we measured the change in GABA content after injection of isoniazid or gabaculine into the median raphe to block synthesis or degradation, respectively.

Major findings

- 1) Turnover of 5HT is under tonic inhibitory GABA-ergic control. GABA agonists increase 5HT turnover, GABA antagonists decrease 5HT turnover.
- 2) 5HT cells are activated by local injection of substance P.
- 3) 5HT cells are also activated by D-Ala-methionine enkephalin amide.
- 4) Injection of an irreversible GABA transaminase inhibitor (gabaculine) to block GABA catabolism causes an increase in GABA content that is rapid in onset and linear for several hours after injection.
- 5) Injection of substance P into the median raphe causes a significant increase in the gabaculine induced GABA accumulation.
- 6) Injection of a GABA synthesis blocker (isoniazid) caused a rapid decrease in GABA content, but the decrease was not exponential, and was brief in duration.
- 7) Lesion of the striato-nigral pathway caused a 80% decrease in GABA content of the substantia nigra, and an 80% decrease in the gabaculine induced GABA accumulation.
- 8) The GABA which accumulates after gabaculine is probably not physiologically active since it does not mimic the effect on serotonergic activity of injection of the GABA agonist muscimol.
- 9) The approach of local injection of gabaculine to irreversibly inhibit GABA breakdown has the advantage over the intraperitoneal or intraventricular route of less possibility of interference due to effects on other pathways, lower toxicity, more rapid onset of action, linear initial rates of accumulation, and greater possibilities of control of concentration in discrete areas.

Significance

New approaches to study GABA should aid us in our understanding of this important inhibitory transmitter. While it remains to be seen whether the estimate of GABA turnover obtained with the use of gabaculine is quantitatively correct, the technique should prove very useful in study of the interaction of GABA with other transmitters. Therapeutically important drugs such as the benzodiazepines (valium) work via GABAergic mechanisms, so studies of how these drugs affect GABA turnover in discrete nuclei may help us to understand some of the details of how these drugs affect brain function.

This project has been terminated.

Publications:

Forchetti, C.M., and Meek, J.L.: Neurotransmitter interactions in the median raphe nucleus. In Usdin, E., Youdim, M.B.H. and Weiner, N. (Eds.): Structure and Function of Monoamine Enzymes. London, Macmillan, 1981, pp. 347-351.

Forchetti, C.M., Marco, E.J., and Meek, J.L.: Serotonin and GABA turnover after injection into the median raphe of substance P and D-Ala-met enkephalin-amide. J. Neurochem. 38: 1336-1341, 1982.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01549-03 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Regulation of imipramine binding sites in rat brain | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | M. L. Barbaccia N. Brunello D. M. Chuang E. Costa | Visiting Fellow Visiting Associate Chemist Chief |
| | | SMRP SMRP SMRP SMRP |
| | | NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.8 | PROFESSIONAL: 1.8 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) We have used rat <u>hippocampus</u> as a model to study the synaptic locations of ³ H-imipramine and ³ H-mianserin binding sites and role in the therapeutic action of antidepressants. Degeneration of <u>5HT</u> terminals with 5,7-dihydroxytryptamine (5,7-DHT) lesion or <u>fimbria-fornix transection</u> led to a decrease in <u>imipramine binding sites</u> but an increase in <u>mianserin binding sites</u> . Lesion with <u>kainic acid</u> decreased the binding sites for <u>mianserin</u> without affecting the binding for imipramine. Hence the majority of imipramine binding sites are located presynaptically while most of the mianserin sites are present postsynaptically in the 5HT synapses. Lesions of 5-HT terminals abolished the <u>down-regulation of β-adrenergic receptors</u> in the hippocampal membranes of rats treated chronically with imipramine or desipramine. Thus an interneuronal system connecting the axons of 5-HT and NE neurons is operating during imipramine-induced β-receptor down-regulation. Following chronic treatments with imipramine or desipramine, the density of imipramine binding sites was decreased and the uptake of ³ H-5HT in the hippocampal slices was facilitated. The disinhibition of the uptake may modify the synaptic function of the interneuron, resulting in down-regulation of β-adrenergic receptors and relief of depression symptoms. | | |

Project Description:

Despite the widespread use of antidepressant drugs in the treatment of depression, the precise mechanism of their therapeutic action remains to be elucidated. Recently, high affinity binding sites specific for typical (^3H -imipramine) and atypical (^3H -mianserin) antidepressant drugs have been described in various brain structures of rat, human and other species. These findings have opened a new avenue to study the molecular mechanisms involved in the therapeutic actions of imipramine and mianserin and to elucidate the biochemical markers of affective disorders. We have used rat hippocampus as a model system to study the synaptic locations of ^3H -imipramine and ^3H -mianserin binding sites. Destruction of 5-HT nerve axons by intragerebral injection of 5,7-dihydroxytryptamine (5,7-DHT) led to a reduction of ^3H -imipramine binding sites by about 60%, whereas the number of the binding sites for ^3H -mianserin was increased by almost 100%. Similar results were obtained when the serotonergic input to the hippocampus was deafferented by an unilateral transection of the fimbria-fornix tract. A depletion of 5-HT stores by chronic treatment with p-chlorophenylalanine (which leaves intact the 5-HT nerve terminals) caused an increase in the density of ^3H -mianserin binding sites without affecting the binding of ^3H -imipramine. Moreover, lesion with kainic acid, which destroys cell body but leaves intact the axon terminals, attenuated the number of ^3H -mianserin binding sites but not of ^3H -imipramine binding sites. These results indicate that the synaptic locations of imipramine and mianserin binding sites are different. A majority of the former is present pre-synaptically while the latter is located post-synaptically in the serotonin synapses in the hippocampus and other brain structures.

Previous studies by Sulser and coworkers (Biochem. Pharmacol. 27:257, 1978) have suggested that desensitization of β -adrenergic receptors in brain after chronic treatments with antidepressants are related to the therapeutic effects of these drugs. To evaluate the role of presynaptic imipramine binding sites in mediating the down-regulation of β -adrenergic receptors, we have performed lesion with 5,7-DHT to degenerate the 5-HT terminals which contain the binding sites for imipramine. This 5,7-DHT lesion was found to prevent the loss of β -receptor binding sites as well as the attenuated responsiveness of adenylate cyclase to β -receptor stimulation in isolated hippocampal membranes of rats treated chronically with imipramine or DMI. These results indicate that 5-HT axons play a permissive role in the drug-induced desensitization of β -adrenergic receptors and that an interneuronal system integrating the axons of 5HT and NE neurons is operative in bringing about this event. We have previously reported that in the hippocampus and cortex of rats treated chronically with imipramine or DMI, the density of imipramine binding sites was decreased, suggesting that these sites are recognized by endogenous modulators or cotransmitters and that imipramine mimics the action of an agonist for these sites. Since imipramine binding sites are related to an regulatory site inhibiting the 5HT uptake (Science 210:1133, 1980), one might expect that 5-HT uptake in chronically treated rats is facilitated due to a disinhibition of the uptake. This view has been supported by our recent finding that the V_{max} of the uptake of ^3H -5-HT into hippocampal slices was enhanced by about 30% after treatment with imipramine (10 mg/kg, i.p. twice daily) for 21 days. Chronic treatment with fluoxetine, another 5-HT uptake blocker which failed to down-regulate β -receptors, did not change this uptake. The increased uptake of 5-HT

after chronic imipramine treatment may result in a reduced amount of 5-HT available in the synaptic cleft, thereby diminishing the transmission of serotonin and modifying the synaptic activity of the proposed interneuronal system. It is our current thinking that the modification of the interneuron is essential for the imipramine-induced down-regulation of β -adrenergic receptors and the therapeutic effect of this drug.

We have also initiated our attempt to investigate the mechanisms of mianserin-induced decrease in the NE-sensitive adenylate cyclase. Lesion with 5,7-DHT failed to prevent the down-regulation of NE-sensitive adenylate cyclase in brain slices induced by chronic mianserin, suggesting that mianserin exerts its effect through a direct interaction with the post-synaptic cells in the 5-HT synapses. It has been reported that mianserin labels the sites of 5-HT₂ receptors. We have found that in rats treated chronically with mianserin, the number of binding sites for 5-HT₂ receptors was decreased whereas the binding sites for ³H-mianserin was unaffected. These results may indicate that mianserin labels a site related but not identical to 5-HT₂ receptors. We propose that mianserin acts on a site that controls the synaptic transmission of 5-HT in a negative manner. Thus mianserin by acting post-synaptically and imipramine and related drugs by acting pre-synaptically may regulate the function of β -adrenergic receptors through a modification of an interneuronal system connecting 5HT and NE nerve terminals. Our current working hypothesis is that depression could involve a deficit in the function of the interneuron and this deficit could be compensated by chronic treatments with antidepressants. These studies may lead to an elucidation of the molecular nature of possible biochemical markers and etiology for depressive state. Further investigation of this project will include: 1) characterization of the interneuron that connects the axons of 5-HT and NE; 2) identification of endogenous substances that recognize the high affinity binding sites for antidepressants and examination of whether the levels of these substances are altered in the CSF of patients with affective disorders.

Publications:

Kinnier, W.J., Chuang, D.M., Gwynn, G., and Costa, E.: Characterization and regulation of high affinity ³H-imipramine binding to rat hippocampal membranes. Neuropharmacology 20: 411-419, 1981.

Brunello, N., Chuang, D.M., and Costa, E.: Difference in the synaptic location of mianserin and imipramine binding sites. Science 215: 1112-1115, 1982.

Brunello, N., Chuang, D.M., and Costa, E.: Characterization of typical and atypical antidepressant recognition sites in rat brain. In Costa, E., and Racagni, G. (Eds.): Typical and Atypical Antidepressants. Advances in Biochemical Psychopharmacology. New York, Raven Press, 1982, vol. 31, pp. 179-184.

Brunello, N., Chuang, D.M., and Costa, E.: Specific binding of ³H-mianserin and ³H-imipramine to structures of rat hippocampus. Europ. J. Pharmacol. 78: 283-284, 1982.

Chuang, D.M., Brunello, N., Kinnier, W.J., and Costa, E.: Regulation of high affinity binding sites for typical and atypical antidepressants in rat brain. In Langer, S.Z., Takahashi, R., and Briley, M. (Eds.): New Vistas in Depression. New York, Pergamon Press, in press.

Brunello, N., Chuang, D.M., and Costa, E.: Use of specific brain lesions to study the site of action of antidepressants. In Langer, S.Z., Takahashi, R., and Briley, M. (Eds.): New Vistas in Depression. New York, Pergamon Press, in press.

Brunello, N., Barbaccia, M.L., Chuang, D.M., and Costa, E.: Down regulation of β -adrenergic receptors following repeated desmethylimipramine injections: Permissive role of serotonergic axons. Neuropharmacology, in press.

Barbaccia, M.L., Brunello, N., Chuang, D.M., and Costa, E.: On the mode of action of imipramine: Relationship between serotonergic axon terminal function and down regulation of β -adrenergic receptors. Neuropharmacology, in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01550-02 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biochemical mechanisms regulated by dopamine D-2 receptors in anterior pituitary | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | P. Onali M. Orianas J. P. Schwartz E. Costa | Visiting Associate Visiting Associate Research Chemist Chief |
| | | SMRP SMRP SMRP SMRP |
| | | NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The interaction of stimulatory and inhibitory receptors at the level of <u>adenylate cyclase</u> has been studied in three systems. In rat <u>anterior pituitary</u> , <u>vasoactive intestinal peptide</u> (VIP) stimulates <u>adenylate cyclase</u> and <u>prolactin</u> release in the mammothrophs. <u>Dopamine</u> can block both of these responses through action at a D-2 receptor. The rat <u>pituitary GH3 cell line</u> provides a single population of cells which also respond to VIP with both <u>adenylate cyclase</u> activation and <u>prolactin</u> secretion. <u>Muscarinic agonists</u> can inhibit both basal and VIP-stimulated <u>adenylate cyclase</u> , as well as <u>prolactin</u> secretion, in this cell line. <u>Muscarinic</u> receptors in rat <u>striatum</u> also inhibit <u>adenylate cyclase</u> and concurrently stimulate a high affinity <u>GTPase</u> , suggesting that inhibitory receptors may affect <u>adenylate cyclase</u> via action on a <u>GTPase</u> . The GH3 cells will allow us to examine this question further in a pure cell population. | | |

Project Description:

Neurotransmitters may modify adenylate cyclase by either stimulating or inhibiting its activity. In a given cell, opposing regulatory inputs may converge on this enzyme system and regulate the rate of formation of cyclic AMP. Thus the identification of these modulators and the study of their mechanism of action constitute a crucial step in the understanding of the transmembrane regulation of adenylate cyclase. These studies have utilized three tissues, rat anterior pituitary, the rat pituitary GH3 cell line, and rat striatum.

Dopamine modulates the adenylate cyclase of rat anterior pituitary by decreasing the response of this enzyme to the stimulatory effect of vasoactive intestinal peptide (VIP), a prolactin-releasing hormone. This original observation led us to investigate the mode of interaction of the dopamine receptors with the adenylate cyclase system of the anterior pituitary. By using primary cultures of rat anterior pituitary, we observed that dopamine was able to inhibit the adenylate cyclase activity stimulated by cholera toxin, a specific activator of the enzyme and an effective prolactin-releasing factor. The inhibitory effect of dopamine was concentration-dependent, required the presence of guanine nucleotides and was blocked by specific dopaminergic antagonists. Because of the GTP dependency and of the inability of dopamine to inhibit the activation of adenylate cyclase by agents which act directly on the catalytic subunit of the enzyme, we concluded that the inhibitory coupling of the dopamine receptors with the adenylate cyclase occurred at the level of the regulatory protein(s) of this enzyme system. One of the functional properties of these proteins is the ability to bind and to hydrolyze GTP by a specific high affinity GTPase. Therefore, the activity of these GTPases could be an expression of the level of involvement of the regulatory proteins in the response of the enzyme system to neurotransmitters.

In rat striatum, the occupancy of muscarinic receptors inhibits the adenylate cyclase activity present in synaptic plasma membranes. These membranes contain a high affinity GTPase activity, which can be detected under the same experimental conditions used for the measurements of adenylate cyclase activity. Therefore we have investigated the effect of the activation of the muscarinic receptors on this GTPase activity and compared these effects with the action of these receptors on the adenylate cyclase. We have found that acetylcholine stimulated the GTPase activity with an apparent EC_{50} of 3 μ M, similar to that found for the inhibition of adenylate cyclase. Acetylcholine increased the V_{max} of the GTPase, without changing the K_m for GTP. Atropine, but not d-tubocurarine, antagonized the stimulation of GTPase by acetylcholine. The hydrolysis of GTP was competitively inhibited by a stable analog of GTP, GMP-PNHP, which does not substitute for GTP in supporting the inhibitory effect of acetylcholine on the adenylate cyclase activity. These results indicate that the activation of GTPase is associated with the inhibitory coupling of the muscarinic receptors to the adenylate cyclase system. We are currently investigating the effect of agents known to modify the a.c. activity on the hydrolysis of GTP.

The rat pituitary tumor cell line, GH3 cells, which secretes prolactin and growth hormone, has receptors for muscarinic agonists and for the vasoactive

intestinal peptide. ACh inhibits prolactin release while VIP stimulates it. We have found that ACh inhibits both basal and VIP-stimulated adenylate cyclase activity, at concentrations comparable to those which affect prolactin secretion. It will be extremely important to investigate whether the inhibitory effect of ACh on the a.c. system correlates with its inhibitory action on PRL release. These studies are not only of interest for elucidating the role of cyclic AMP in the release of pituitary hormones, but also for the demonstration of an interaction of different neurotransmitters in the regulation of cell function.

Publications:

Onali, P., Schwartz, J.P., and Costa, E.: Stimulation of dopamine receptors inhibits vasoactive intestinal peptide stimulation of adenylate cyclase in rat anterior pituitary. Proc. Natl. Acad. Sci. USA 78: 6531-6534, 1981.

Onali, P., Schwartz, J.P., and Costa, E.: Inhibition of VIP-sensitive adenylate cyclase by dopamine in rat anterior pituitary. In Jacob, J., Kuriyama, K., Segawa, T., and Yamamura, H.J. (Eds.): Molecular Pharmacology of Neurotransmitter Receptor Systems, in press.

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|--|--|--|------|--------------|---------|------|------|--------|-----------------|-----------------|------|------|--|-----------|-----------------|------|------|--|----------|-------|------|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01551-02 SMRP | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Is insulin a neuromodulator in the central nervous system? | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">D. M. Chuang</td> <td style="width: 20%;">Chemist</td> <td style="width: 15%;">SMRP</td> <td style="width: 15%;">NIMH</td> </tr> <tr> <td>Other:</td> <td>M. L. Barbaccia</td> <td>Visiting Fellow</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>P. Panula</td> <td>Visiting Fellow</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>E. Costa</td> <td>Chief</td> <td>SMRP</td> <td>NIMH</td> </tr> </table> | | | PI: | D. M. Chuang | Chemist | SMRP | NIMH | Other: | M. L. Barbaccia | Visiting Fellow | SMRP | NIMH | | P. Panula | Visiting Fellow | SMRP | NIMH | | E. Costa | Chief | SMRP | NIMH |
| PI: | D. M. Chuang | Chemist | SMRP | NIMH | | | | | | | | | | | | | | | | | | |
| Other: | M. L. Barbaccia | Visiting Fellow | SMRP | NIMH | | | | | | | | | | | | | | | | | | |
| | P. Panula | Visiting Fellow | SMRP | NIMH | | | | | | | | | | | | | | | | | | |
| | E. Costa | Chief | SMRP | NIMH | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Molecular Neurobiology | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.60 | PROFESSIONAL: 0.60 | OTHER: 0 | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Rat brain contains <u>insulin-like peptide</u> and <u>binding sites recognized by insulin</u> . Using <u>olfactory bulb slices</u> we have found that cAMP content can be increased by either insulin or <u>dopamine</u> (DA). The insulin-dependent accumulation of cAMP was facilitated by <u>sulpiride</u> but was unaffected by <u>haloperidol</u> . Simultaneous addition of insulin and DA failed to increase cAMP when <u>GppNhp</u> was present, indicating that insulin interacts with DA and that insulin may be a <u>neuromodulator</u> for DA. We are now studying the synaptic location and transcriptional regulation of <u>mRNA's</u> for insulin-like peptide using a <u>cDNA</u> probe prepared from a bacterial clone synthesizing rat proinsulin. Since <u>internalization</u> of insulin may be a mechanism that explain interactions between DA and insulin in rat brain, we have also studied insulin internalization using <u>frog erythrocytes</u> as a model system. Using erythrocytes incubated with ¹²⁵ I-insulin, we have found a temperature dependent and energy required accumulation of radioactivity in the intracellular fraction. Morphologically we were also able to visualize insulin internalization using cells incubated with <u>rhodamine-labeled with insulin</u> . Studies are in progress to clarify the molecular mechanisms and functional significant of this insulin internalization. | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

It has been reported that rat brain contains insulin-like peptide (ILP) and binding sites that recognize insulin. However the function of ILP and insulin binding sites in CNS was unknown. Our working hypothesis was that insulin in brain may modulate synaptic function. Since olfactory bulb is one of the areas richest in ILP and contains high density of binding sites, we have used this brain region as a model system to study whether insulin modifies central synaptic mechanisms.

Immunohistochemistry has shown that olfactory bulb contains dopaminergic cell bodies located among the periglomerular and tufted cells. We have therefore examined whether olfactory bulb contains dopamine (DA)-dependent adenylyl cyclase. Using 300 μ slices of the olfactory bulb. We have found that DA in concentration 3×10^{-5} M or greater increased the cAMP content of olfactory bulb slices. Monolateral bulbectomy increased the responsiveness of adenylyl cyclase in the contralateral olfactory bulb to DA stimulation. The cAMP accumulation elicited by DA was partially inhibited by haloperidol (5×10^{-6} M), while was facilitated by sulpiride (5×10^{-6} M) and IBMX (5×10^{-6} M). Insulin, which by itself did not affect the cAMP content when added to slices of frontal cortex and striatum, was also able to increase in a concentration depending manner (from 3×10^{-7} M to 1.8×10^{-6} M) the cAMP content of olfactory bulb slices. This insulin action was unaffected by haloperidol (5×10^{-6} M), but was facilitated by sulpiride (5×10^{-6} M) and IBMX (5×10^{-6} M). Monolateral bulbectomy facilitated the responsiveness in contralateral bulb to insulin in terms of cAMP accumulation. Moreover, when the maximal effective doses of DA (10^{-4} M) and insulin (10^{-6} M) were applied together in the same olfactory bulb slices preparation we would no longer detect the increase of cAMP content elicited by these two hormones as when given separately. These insulin-induced effects were facilitated by the presence of GppNHP in the reaction mixtures. Thus our results suggest that insulin and DA are interacting at the level of GTP binding protein in the olfactory bulb of rat. The neuronal location of insulin or ILP in olfactory bulb and the molecular mechanisms whereby dopaminergic receptors and insulin receptors interreact are currently under our investigation.

Whether ILP in rat brain is synthesized in situ or originated from the pancreas is of controversy based on the results obtained with immunochemical methods (Howrankova et al., PNAS 75:5735, 1978; Yallow and coworkers, PNAS 78:4576, 1981). Recently Villa-Komaroff and coworkers have used a cDNA probe for rat proinsulin from an insulin-producing tumor of the pancreas. They have found that there is one and possible two mRNA's in adult rat brain that bind to the cDNA insulin probe but they are not insulin mRNA's. We have obtained from Dr. Villa-Komaroff a bacteria clone that synthesizes the cDNA probe for proinsulin. Using this cDNA probe we are attempting to address the following questions: Is the mRNA for ILP co-present with DA in the periglomerular and tufted cells in the olfactory bulb? Is the transcription of mRNA for ILP under the transsynaptic regulation? Does ILP function as a neuromodulator or co-transmitter for the synaptic transmission? These questions are now under our investigation.

It has been shown in different systems that insulin can be internalized following its binding to cell surface receptors. It has also been speculated

that this insulin internalization may be essential for some long term action of the hormone such as enhanced gene expression. Also in brain the action of insulin may require internalization. We used the frog erythrocytes as a model system to characterize these "post-receptor" events. We found that the frog erythrocytes can bind insulin in a temperature-dependent manner; the binding is biphasic at 30°C and monophasic at 4°C. At 30°C the two binding sites have a Kd of 0.6 and 18 nM, respectively; at 4°C the Kd is 22 nM. Incubation of erythrocytes for 1 h at 30°C with different concentrations of insulin (33, 330 nM) caused a decrease in Bmax by about 30% with no change in Kd. Most of the insulin specifically bound to frog erythrocytes at 4°C could be removed by treatment with 0.2N acetic acid/0.5M NaCl. In contrast after 30 minutes of incubation at 30°C the insulin was incorporated into the erythrocytes by a different mechanism and could not be released anymore by the above mentioned procedure. Since this mild acid treatment has been shown to remove selectively the ligand bound to surface receptors, the tenacious binding may therefore reflect the internalization of insulin molecules. The insulin internalization occurring at 30°C could be inhibited by dinitrophenol in a dose dependent manner, suggesting the involvement of energy-requiring steps. Both the detector populations found at 30°C seem to be involved in the internalization of insulin. It is now important to characterize the insulin internalization. We plan to pursue this goal biochemically and morphologically. Biochemically we have used Percoll continuous gradient and gel filtration. The results suggest that the insulin internalized is partially associated with lysosomes and it might be degraded by lysosomal enzymes. Now we are studying whether degradation products of internalized insulin have a physiological role. Morphologically we have used frog erythrocytes incubated with insulin labeled with rhodamine to visualize directly the internalization of insulin molecules. Our preliminary results have shown a receptor-mediated appearance of punctated spots of fluorescence in some of the erythrocytes incubated with rhodamine-insulin. The punctated fluorescent spots may reflect the presence of clustered complexes of insulin and its receptor in endocytic vesicle such as the receptosome. Studies are in progress to obtain direct evidence in support of this conclusion.

In summary the aim of this project is to study the role of insulin or insulin-like peptide as a brain cotransmitter modulating the synaptic transmission of known messengers and to elucidate the molecular mechanisms mediating the physiological and pharmacological function of insulin.

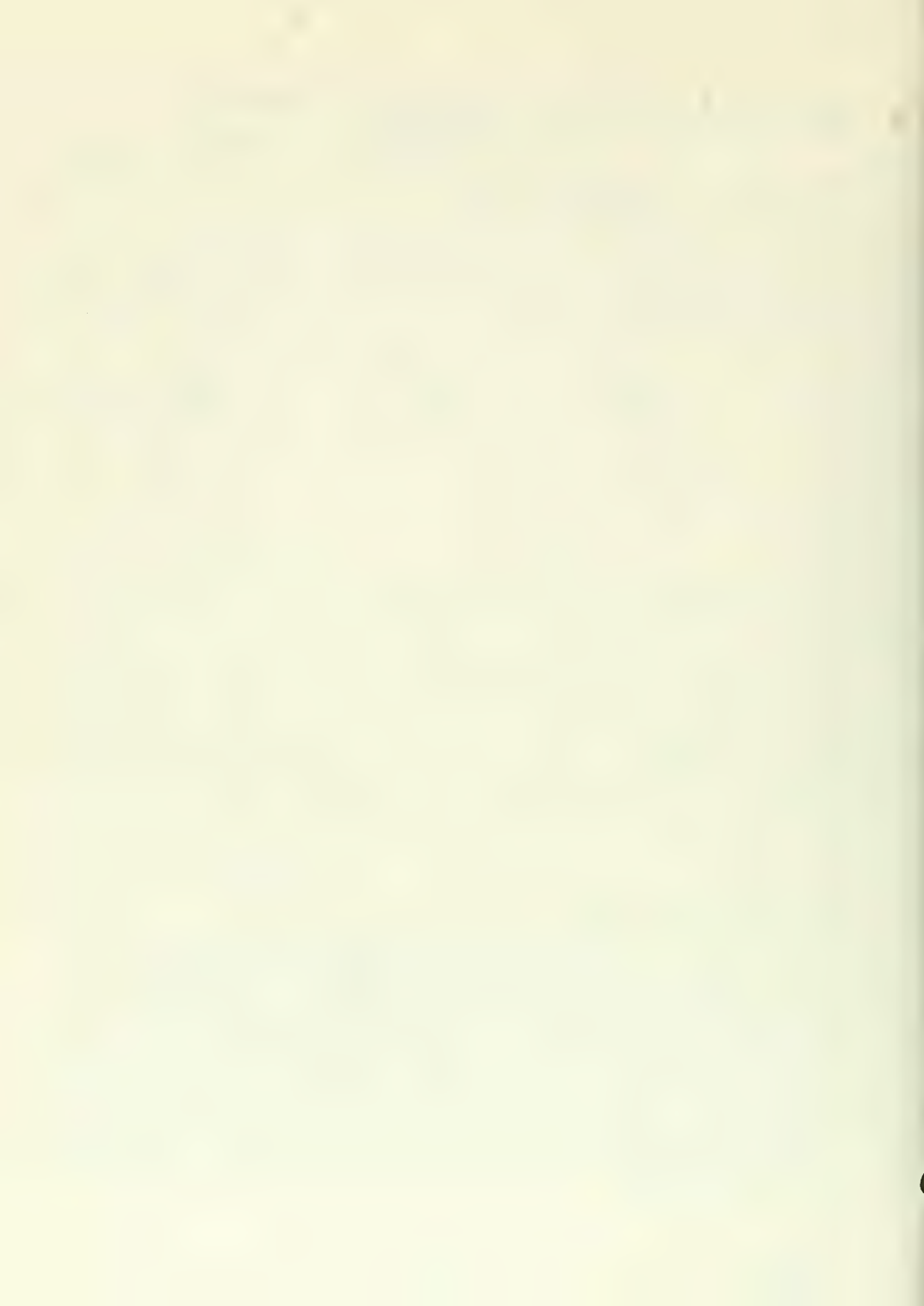
Publications:

Barbaccia, M.L., Chuang, D.M., and Costa, E.: Is insulin a neuromodulator? In Regulatory Peptides: Functional and Pharmacological Aspects. Advances in Biochemical Psychopharmacology. New York, Raven Press, in press.

Chuang, D.M., Barbaccia, M.L., Brunello, N., and Kinnier, W.J.: Receptor regulation: An overview. In Hanin, I. (Ed.): Dynamics of Neurotransmission. New York, Raven Press, in press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01552-02 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Agonist and antagonist of benzodiazepine receptors | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | M. G. Corda A. Guidotti E. Costa | Guest Worker Chief Chief |
| | | SMRP SMRP-N SMRP |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Neuroendocrinology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 1.2 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Drug binding to recognition sites for endogenous ligands can act as agonists and antagonists. An <u>imidazobenzodiazepine</u> derivative, RO 15-1788 is the first representative of specific <u>benzodiazepine</u> antagonists. However since anxiolytic benzodiazepines probably act as antagonists, the RO 15-1788 may be acting as an agonist, that is it mimics the endogenous agonist. Studies with isoniazid show that benzodiazepines antagonize the convulsant action of isoniazid, while RO 15-1788 facilitates these convulsions. It has been reported that β -carbolines bind to benzodiazepine recognition sites in brain. These drugs, however, differ from benzodiazepines because they are convulsant or proconvulsant. Behavioral studies with β -carbolines indicate that these drugs unveil latent conflict behavior in rat. The possibility that β -carbolines and RO 15-1788 mimic the action of an endogenous anxiogenic agent is at present under investigation. | | |



studying the possible existence of endogenous ligands for benzodiazepine receptors. Clinical understanding of these problems will help to elucidate pathological anxiety and etiology of convulsive diseases and to predict possible therapeutic applications of agonist and antagonist of benzodiazepine receptors.

Publication:

Corda, M.G., Costa, E., and Guidotti, A.: Specific proconvulsant action of an imidazobenzodiazepine (RO 15-1788) on isoniazid convulsions. Neuropharmacology 21: 91-94, 1982.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01553-02 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Biosynthesis of met ⁵ - and leu ⁵ -enkephalins in bovine adrenal medulla | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | I. Lindberg H.-Y.T. Yang E. Costa | Staff Fellow Pharmacologist Chief SMRP SMRP SMRP |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.1 | PROFESSIONAL: 1.1 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) In this study, the enzymatic biosynthesis of met ⁵ -enkephalin from endogenous precursors was investigated. A trypsin-like enzyme was partially purified from bovine adrenal chromaffin granules through the use of affinity chromatography. This enzyme preparation was able to generate met ⁵ -enkephalin from endogenous substrate(s). Met ⁵ -enkephalin production was not inhibited by sulfhydryl reagents such as p-chloromercuriphenyl sulfonate nor stimulated by dithiothreitol, suggesting that this enzyme is not a lysosomal enzyme such as cathepsin B. Enzymatic activity was strongly inhibited by several trypsin inhibitors including soybean trypsin inhibitor, aprotinin, and diisopropylfluorophosphate. These results imply that this adrenal enzyme is not a protease of lysosomal origin but is a chromaffin granular enzyme capable of generating enkephalin. The physiological role of enkephalin in adrenal is still unclear. The identification of this enkephalin generating enzyme will now enable us to study the regulation of enkephalin system in the adrenal and then, in turn, explore its physiological role. The enzyme will be further purified, characterized and a specific method will be developed to study the regulation of the adrenal enkephalin system. | | |

Project Description:

The adrenal medulla contains large quantities of high molecular weight forms of enkephalin; some of these peptides may represent precursors to met- and leu-enkephalin. We have previously shown that adrenal medullary chromaffin granules contain a trypsin-like enzyme which is capable of generating met-enkephalin from endogenous high molecular weight precursor(s). Using a soluble protein fraction prepared from lysed chromaffin granules, we have partially purified this enzymatic activity by affinity chromatography on soybean trypsin inhibitor coupled to Sepharose. Approximately 0.2% of the lysate protein applied to the affinity column was retained after extensive washing of the column with buffer. This protein was eluted with 0.25 M acetic acid and, following neutralization and concentration, used as the enzyme source; material which was not retained by the affinity column was concentrated, heated, and used as the substrate source. It was shown that the partially purified enzyme is able to generate met-enkephalin as well as other low molecular weight enkephalin-immunoreactive peptides from high molecular weight substrate(s); Peptide F could also serve as the substrate for the enzyme preparation to yield low molecular weight enkephalin-immunoreactive peptides. The generation of enkephalin-immunoreactivity was found to be dependent on the length of incubation and the substrate concentration. Production of met-enkephalin-immunoreactivity was not inhibited by sulfhydryl reagents but was inhibited by soybean trypsin inhibitor, trasylol, and diisopropylfluorophosphate, suggesting that the enzyme is a serine protease and not a cathepsin of lysosomal origin. This conclusion is further supported by the finding that the pH optimum of the enzymatic activity was 7.5-8.0.

The results, taken together, suggest that this adrenal enzyme is a chromaffin granular enzyme and probably is an enzyme involved in the biosynthesis of enkephalin in adrenal. Further purification, characterization and development of a specific enzyme assay method is in progress. The regulation of this enkephalin generating enzyme will be explored pharmacologically.

Publication:

Lindberg, I., Yang, H.-Y.T., and Costa, E.: An enkephalin-generating enzyme in bovine adrenal medulla. Biochem. Biophys. Res. Commun. 106: 186-193, 1982.

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|--|--|--|------|-----------|--------------|------|------|--------|---------------|--------------------|------|------|--------------|----------------|------|------|-------------|--------------------|------|------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01554-02 SMRP | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Met ⁵ -enkephalin system in pituitary: Heroin addicts vs controls | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">S. Govoni</td> <td style="width: 25%;">Guest Worker</td> <td style="width: 15%;">SMRP</td> <td style="width: 20%;">NIMH</td> </tr> <tr> <td rowspan="3">Other:</td> <td>L. G. Harsing</td> <td>Visiting Associate</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td>H.-Y.T. Yang</td> <td>Pharmacologist</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td>J. Kleinman</td> <td>Staff Psychiatrist</td> <td>SMRP</td> <td>NIMH</td> </tr> </table> | | | PI: | S. Govoni | Guest Worker | SMRP | NIMH | Other: | L. G. Harsing | Visiting Associate | SMRP | NIMH | H.-Y.T. Yang | Pharmacologist | SMRP | NIMH | J. Kleinman | Staff Psychiatrist | SMRP | NIMH |
| PI: | S. Govoni | Guest Worker | SMRP | NIMH | | | | | | | | | | | | | | | | |
| Other: | L. G. Harsing | Visiting Associate | SMRP | NIMH | | | | | | | | | | | | | | | | |
| | H.-Y.T. Yang | Pharmacologist | SMRP | NIMH | | | | | | | | | | | | | | | | |
| | J. Kleinman | Staff Psychiatrist | SMRP | NIMH | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Adult Psychiatry Branch | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology SECTION Molecular Neurobiology INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.5 | PROFESSIONAL: 0.5 | OTHER: 0 | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Very high contents of <u>met⁵-enkephalin-like peptides</u> were found in <u>pituitaries</u> of many species if animals were killed by decapitation. Furthermore, <u>met⁵-enkephalin immunoreactivity</u> in pituitary was found to consist of high (MW <u>~1800</u>) and low (MW about 600) molecular weight <u>enkephalin-like peptides</u> . Interestingly, one of the high molecular weight forms was found to be much lower in <u>pituitaries of heroin addicts</u> in comparison to controls. The molecular mechanism <u>underline this heroin induced modification</u> in <u>met⁵-enkephalin immunoreactivity</u> still remains to be established. | | | | | | | | | | | | | | | | | | | | |

Proposed Course:

This project has been terminated.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 01555-02 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Enkephalin metabolism | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | An-Zhong Zhang H.-Y.T. Yang E. M. Majane J. Tang | Visiting Fellow Pharmacologist Chemist Visiting Fellow SMRP SMRP SMRP SMRP NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.9 | PROFESSIONAL: 1.9 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The metabolism of <u>opioid peptides</u> , <u>met⁵-enkephalin</u> and <u>met⁵-enkephalin-arg⁶-phe⁷</u> (YGGFMRF) was studied both in vitro and in vivo. Both met ⁵ -enkephalin and YGGFMRF are readily hydrolyzed by <u>dipeptidyl carboxypeptidase</u> . These two enzyme activities, YGGFMRF hydrolyzing activity and enkephalin inactivating activity (enkephalinase), can be differentially inhibited by specific inhibitors <u>capto-</u> <u>pril</u> (for YGGFMRF hydrolysis) and <u>thiorphan</u> (for enkephalinase). Using these inhibitors, the possible role of the dipeptidyl carboxypeptidase in metabolism of met ⁵ -enkephalin and YGGFMRF were studied in vivo. The thiorphan injected intracerebrally into mice increased the striatal content of met ⁵ -enkephalin and also the jump latency from the hot plate. The captopril administration intracerebrally, on the other hand, elevated the striatal content of YGGFMRF in the mice. In the rats, this treatment was found to increase the duration of <u>acupuncture</u> induced <u>analgesia</u> and also the striatal YGGFMRF content by 100%. The results indicate that activity of endogenous opioid system can be activated pharmacologically. The effect of the drug, inhibitor of dipeptidyl carboxypeptidase, on endogenous YGGFMR will be continuously explored in area such as spinal cord, pituitary, lung and heart. | | |

Project Description:

Previously, we have observed that both enkephalin and $\text{met}^5\text{-enkephalin-arg}^6\text{-phe}^7$ (YGGFMRF) can be hydrolyzed by dipeptidyl carboxypeptidase *in vitro*. In searching for a tool to explore the possible role of the dipeptidyl carboxypeptidase in opioid peptide metabolism, effects of various dipeptidyl carboxypeptidase inhibitors on YGGFMRF hydrolyzing activity and enkephalin inactivating activity (enkephalinase) were evaluated. Captopril inhibited the YGGFMRF hydrolyzing activity with IC_{50} of $5 \times 10^{-8} \text{ M}$ but was totally inactive against enkephalinase. The other angiotensin converting enzyme inhibitor, SQ 20881, also markedly inhibited the YGGFMRF hydrolyzing activity with IC_{50} of $3 \times 10^{-7} \text{ M}$, while it reduced the enkephalinase only by about 20% at 10^{-6} M . Acetthiorphan, on the other hand, efficiently depressed the enkephalinase with an IC_{50} of $3 \times 10^{-7} \text{ M}$ but showed little effect on YGGFMRF hydrolyzing activity. A similar differential inhibitory property was also observed for the other enkephalinase inhibitor, thiorphan. With these specific inhibitors, thiorphan and captopril, we have investigated the possible participation of the dipeptidyl carboxypeptidase in the metabolism of YGGFMRF and $\text{met}^5\text{-enkephalin}$ *in vivo*.

Thiorphan, injected intracerebrally into mice, inhibited the enkephalinase in striatum by 90 to 80% during the first hour. This decrease in enkephalinase was accompanied by 30% increase in striatal $\text{met}^5\text{-enkephalin}$ content. The thiorphan treatment also increased the jump latency from the 54°C hot plate, and this effect was reversed by pretreatment with 5 mg/kg naloxone. The results seem to support the view that thiorphan protects endogenous $\text{met}^5\text{-enkephalin}$ from inactivation by enkephalinase and that the accumulation of $\text{met}^5\text{-enkephalin}$ near the receptor may elicit the antinociceptive response.

Captopril injected intracerebrally into mice inhibited the angiotensin converting enzyme markedly and concomitantly elevated the striatal YGGFMRF content by a small degree. The maximum increase of YGGFMRF was observed 30 min after the captopril treatment and no increase in $\text{met}^5\text{-enkephalin}$ content was observed. These results seem to suggest that the captopril sensitive dipeptidyl carboxypeptidase may play a role in, *in vivo*, inactivation of YGGFMRF in mice. It should be noted that the similar effect of captopril was not observed in the rat. However, if rats were treated with captopril and then subjected to electroacupuncture, the striatal content of YGGFMRF but not that of $\text{met}^5\text{-enkephalin}$, was doubled 30 min after the captopril injection and 15 min after the acupuncture. The acupuncture or captopril treatment alone exerted totally no effect on the striatal content of YGGFMRF. A prolongation of the duration of acupuncture analgesia was also observed in rats treated with captopril and electro-acupuncture and this effect was reversed by pretreatment of the animal with naloxone. These results, taken together, seem to suggest that YGGFMRF neuronal activity may be increased during acupuncture and concomitant inhibition of YGGFMRF inactivation *in vivo* consequently resulted in the elevation of YGGFMRF content.

The results strongly indicate that the endogenous opiate peptide, YGGFMRF, is metabolized by a specific enzyme and inhibition of this enzyme can lead to activation of YGGFMRF system *in vivo*. Based on this study, drugs may be designed to modify the function of the opiate peptide, YGGFMRF, and, in turn,

to learn more about the possible role of the endogenous opioid system in psychiatric disorder.

We are planning to continue search for efficient drugs which may be useful in modulating the endogenous opioid system in vivo.

Publications:

Yang, H.-Y.T., Majane, E., and Costa, E.: Conversion of [met⁵]-enkephalin-arg⁸-phe⁷ to met⁵-enkephalin by dipeptidyl carboxypeptidase. Neuropharmacology 20: 891-894, 1981.

Yang, H.-Y.T., Majane, E., and Costa, E.: Conversion of met⁵-enkephalin-arg⁶-phe⁷ to met⁵-enkephalin by dipeptidyl carboxypeptidase. In Advances in Endogenous and Exogenous Opioids. Proceedings of the International Narcotic Research, Kyoto, Japan, July 26-30, 1981, Kodansha Ltd., Tokyo, Japan.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01556-02 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Release of endorphins | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | L. Harsing H.-Y.T. Yang E. Costa J. Del Rio | Visiting Associate Pharmacologist Chief Guest Worker |
| | | SMRP SMRP SMRP SMRP |
| | | NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.4 | PROFESSIONAL: 0.4 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) In this study, the mechanism involved in the anorectic effect of <u>d-fenfluramine</u> and <u>CM 57 277</u> was investigated. Repeated injections of these two <u>anorectic drugs</u> resulted in elevation of hypothalamic <u>met⁵-</u> , <u>leu⁵-enkephalin</u> and <u>β-endorphin</u> . This increase is associated with reduction in body weight. The effect of these two anorectics can be reversed by metergoline, a serotonin receptor antagonist. The results suggest that decreased utilization of hypothalamic opioid peptides caused by a facilitation of serotonergic transmission may be responsible for the anorectic action of fenfluramine and CM 57 277 but not that of amphetamine. Met ⁵ -enkephalin release was also investigated in this study. The interaction between serotonergic and endogenous opioid system and the importance of endogenous opioid peptide in eating behavior are well demonstrated in this study. These findings may aid in understanding the abnormal eating behavior. The interaction of met ⁵ -enkephalin and other transmitter, substance P, was also investigated in this study. Met ⁵ -enkephalin can be released from periaqueductal grey slices by substance P suggesting that substance P analgesia may be mediated by opioid peptide. The release of opioid peptide(s) from periaqueductal grey slices will be further characterized. | | |

Project Description:

Consomatory behavior appears to be under inhibitory control of serotonergic neurons which project from the dorsal raphe nucleus to the amygdala and hypothalamus. Recently, accumulating evidence suggests that endogenous opioid peptides may be involved in the regulation of eating behavior. In order to explore the possible interaction between serotonergic and endorphinergic actions in regulation of consumatory behavior, we have studied the effect of d-fenfluramine and CM 57 277, two drugs which increase serotonergic function, on hypothalamic content of met⁵-enkephalin and β -endorphin.

In rats treated for 5 days with d-fenfluramine (15 mg/kg/day) and CM 57 277 (4-amino-6-chloro-2-pyridyl)-1-piperidine HCl (20 mg/kg/day), two drugs which release serotonin, the hypothalamic met⁵-enkephalin and β -endorphin contents were found to be elevated. This elevation was accompanied by a marked decrease of body weight. Metergoline (2x2.5 mg/kg/day), a serotonin receptor antagonist, and p-chlorophenylalanine (PCPA 100 mg/kg/day), a serotonin biosynthesis inhibitor, abolished the accumulation of both met⁵-enkephalin and β -endorphin in the hypothalamus elicited by repeated injections of d-fenfluramine. The content of other hypothalamic neuropeptides, such as cholecystokinin and substance P, were not affected by chronic d-fenfluramine treatment. In striatum, a transient elevation of met⁵-enkephalin was observed but this effect was not reversed by metergoline. In frontal cortex and pituitary, the met⁵-enkephalin contents were unchanged by d-fenfluramine or CM 57 277 treatment. A repeated treatment with naloxone failed to alter the met⁵-enkephalin or β -endorphin content of hypothalamus. The results suggest that an accumulation of hypothalamic met⁵-enkephalin and β -endorphin may participate in the action of these anorectic drugs which facilitate serotonergic transmission. Since naloxone which blocks met⁵-enkephalin and β -endorphin receptors is anorectic, we suggest that one of the cause for accumulation of met⁵-enkephalin and β -endorphin could be a decreased release due to the increased serotonergic tone.

The results further support the role of endogenous opioid peptides in eating behavior. The finding may aid in investigation of abnormal eating behavior.

The release of met⁵-enkephalin from brain slices was also investigated in this study. Periaqueductal gray was found to release met⁵-enkephalin when it was perfused with Krebs-bicarbonate buffer containing 10^{-6} M substance P. The result suggests that the analgesic effect of substance P may be mediated by endogenous opioid peptide. The interaction of endogenous opioid peptide with other transmitter in periaqueductal grey will be further studied.

Publications:

Harsing, L.G., Yang, H.-Y.T., Govoni, S., and Costa, E.: Elevation of met⁵-enkephalin and β -endorphin hypothalamic content in rats receiving anorectic drugs: Differences between d-fenfluramine and d-amphetamine. Neuropharmacology 21: 141-145, 1982.

Harsing, L.G., Yang, H.-Y.T., and Costa, E.: Evidence for a GABA mediation in the benzodiazepine inhibition of the release of met⁵-enkephalin elicited by depolarization. J. Pharmacol. Exp. Ther., in press.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01557-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Immunohistochemical localization of neurotransmitters in the septal complex | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | P. Panula A. V. Revuelta D. L. Cheney | Visiting Fellow Visiting Associate Chief SMRP SMRP SMRP-M NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Pharmacodynamics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.6 | PROFESSIONAL: 1.3 | OTHER: 0.3 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Glutamate decarboxylase (GAD)</u>, a specific marker of GABAergic neurones was found to be widely distributed in the <u>septum</u> of the rat. Immunoreactive fibres and terminals were found in all parts of the <u>lateral septal nucleus</u>. GAD positive neuronal cell bodies were most numerous in the <u>medial septal nucleus</u> and the nucleus of the <u>diagonal band</u>. A dense network of <u>met-enkephalin immunoreactive fibres</u> and <u>terminals</u> was seen in the intermediate part of the lateral septal nucleus. In colchicine-injected animals, numerous met-enkephalin-immunoreactive cell bodies were found in the dorsal, intermediate and ventral parts of the lateral septal nucleus. No cell bodies exhibiting <u>β-endorphin-like immunoreactivity</u> were found in the septal complex, but the basal parts of both lateral and medial septum contained <u>β-endorphin immunoreactive varicose fibres</u>. The results indicate that a large group of GABAergic cells is located in medial septal regions and the terminal fields of septal GABAergic system are mainly in the lateral septum. The enkephalin-containing neuronal system in the septum most likely consists at least partly of septal interneurons whereas the <u>β-endorphin system</u> most probably comes from septal afferents. </p> | | |

Project Description:

The aim of this study was to reveal the distribution of neurotransmitters and neuropeptides in the septal complex which play a major role in the regulation of the function of the limbic system.

Specific antibodies were produced in rabbits against different neuropeptides and an antiserum against glutamate decarboxylase was obtained from Dr. J.-Y. Wu (Baylor College of Medicine, Houston, Texas). The rats were perfused through the left ventricle with buffered formalin, the brains were removed and washed before sectioning with a cryostat. The sections were first incubated with the specific antiserum then washed and processed for either indirect immunofluorescence using fluorescein or rhodamine coupled anti-rabbit antibodies or for peroxidase/antiperoxidase procedure.

GABAergic neurones were found to be confined to the medial septal nucleus, nucleus of the diagonal band and septofimbrial nucleus. Only scattered small cells were seen in the lateral septal nucleus. Thus, GABAergic neurones in the septum are located in the same areas with cholinergic neuronal systems.

There is a large population of enkephalin-immunoreactive neurones in the lateral septal nucleus, one prominent group being localized in the dorsal part of the nucleus. The intermediate part of lateral septal nucleus contains the largest population of enkephalin-immunoreactive cells and scattered cells are also found in the ventral part of the nucleus. The bed nucleus of the stria terminalis also contains numerous cells. Only few single enkephalin-immunoreactive neurones are located in the medial septal nucleus and nucleus of the diagonal band.

β -Endorphin-immunoreactive varicose fibers enter the basal parts of the septal complex from below, most probably from medial hypothalamus where the cell bodies are known to be located. No cell bodies are found in the septal complex.

Substance P is widely distributed in the septum and immunoreactive fibers and terminals are most numerous in the intermediate part of the lateral septal nucleus.

The results indicate that the medial septal complex which is known to send a cholinergic pathway to the hippocampus also contains a population of GABAergic cells. Only few enkephalin-immunoreactive fibres enter this area and enkephalin-immunoreactive terminals are few in number. β -Endorphin-immunoreactive fibers are present in the basal parts of the medial septal complex.

The lateral septal nucleus is rich in both enkephalin-immunoreactive terminals and cell bodies. It remains to be studied whether sources other than hypothalamoseptal enkephalin-containing system and septal enkephalin cells contribute to this system.⁵ When antiserum against a putative enkephalin precursor met-enkephalin-Arg⁶-Phe⁷ was used, fewer cells exhibited immunoreactivity but the dense plexus of immunoreactive fibers and terminals in the intermediate part of the lateral septum was seen. The terminal network of Met⁵-enkephalin-Arg⁶-Phe⁷-immunoreactive fibers in the lateral septum was one of

the densest found in the whole CNS and this region might be suitable for future studies on the role of the heptapeptide either as an enkephalin precursor or neuromodulator.

The septum plays a major role in the modulation of the function of the limbic system, especially by modulating the function of the septo-hippocampal cholinergic pathway which is involved in learning. As a result of this study it appears that the peptidergic system in the septal complex is more extensive than has been thought. Also, the major inhibitory transmitter GABA appears to be used by septal cells.

The exact localization of peptides in relation to cholinergic and GABAergic systems in the septum is necessary for better understanding of the behavioral and motivational functions of the septum as well as disease states which involve the septum. The research in progress will lead to the characterization of individual peptidergic systems in the limbic system and will involve pharmacological studies of these peptidergic systems to elucidate their regulatory, motivational and memory functions.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01558-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Immunohistochemical studies on neuropeptides in the central and peripheral nervous system | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: P. Panula Other: H.-Y.T. Yang D. L. Cheney M. Economou- Hadjiconstantinou E. Costa | Visiting Fellow Pharmacologist Chief Guest Worker Chief | SMRP SMRP SMRP-M SMRP SMRP NIMH NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Pharmacodynamics | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.9 | PROFESSIONAL: 0.9 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Antisera against <u>bombesin</u> , <u>substance P</u> , <u>methionine enkephalin</u> and <u>met⁵-enkephalin-Arg⁶-Phe</u> were used to study the distribution of these <u>peptides</u> in the <u>brain</u> , <u>spinal cord</u> and <u>peripheral organs</u> . Bombesin was found in several cell groups in the brain. One of the major groups in the <u>paraventricular nucleus</u> of the hypothalamus did not exhibit <u>substance P-like immunoreactivity</u> though in several other areas like the <u>nucleus tractus solitarius</u> , <u>dorsal parabrachial nucleus</u> and <u>dorsolateral tegmental nucleus</u> immunoreactivity was found in the same cell groups. In the periphery, bombesin-like immunoreactivity was found in the spinal sensory ganglia, adrenal medulla and nerve fibres innervating the lung. Met ⁵ -enkephalin-Arg ⁶ -Phe ⁷ -like immunoreactivity was found in the same areas of the brain as met-enkephalin. | | |

Project Description:

The aim of this study was to localize new neuropeptides in the central and peripheral nervous system and to reveal the interrelationships of these neuronal systems with previously characterized pathways and target organs.

Antiserum against bombesin was produced and characterized and subsequently used to localize bombesin-like immunoreactivity (BN-LI) in the brain and spinal cord of the rat. In general, the distribution of BN-LI was similar to substance P. However, in the paraventricular nucleus of the hypothalamus the cell group exhibiting BN-LI did not show substance P-like immunoreactivity. Other areas containing BN-LI included the interpeduncular nucleus, central gray, nucleus tractus solitarii and the trigeminal complex. In the spinal cord, a dense network of fibers and terminals exhibited BN-LI in the layer II of the posterior horn. Terminals in the layer VII surrounded large motoneurons and scattered fibers were seen in other areas as well. Rhizotomy at lower lumbar and sacral levels diminished immunoreactivity in the posterior horn considerably but did not abolish BN-LI in the anterior horn. Spinal sensory ganglia contained BN-LI cells, but the number of immunoreactive cells was lower than the number of substance P-positive cells. Thus, the BN-LI in the spinal cord originates from sensory ganglia and partly from another source, probably spinal interneurons, since transection of the spinal cord at mid-thoracic level did not abolish the staining.

Met⁵-enkephalin-Arg⁶-Phe⁷-like immunoreactivity was widely distributed in the brain and it was found in the same areas with met-enkephalin. The antiserum used did not have cross reactivity with met-enkephalin. Cell bodies were found in the striatum, lateral septal nucleus, stria terminalis, olfactory tubercle, in several regions of the hypothalamus, inferior collicle and several pontine and medullary regions. In the hippocampus, the mossy fiber system was very weakly positive whereas the fibres entering from entorhinal cortex showed strong immunoreactivity. In the spinal cord, the substantia gelatinosa of the posterior horn was the most intensively stained regions with numerous fibers and a few cell bodies. Terminals were also seen in the anterior horn around the motoneurons.

In the adrenal medulla, numerous nerve terminal-like structures and some chromaffin cells exhibited Met⁵-enkephalin-Arg⁶-Phe⁷-like immunoreactivity. After adrenal denervation through the splanchnic nerve immunoreactivity in the nerve terminals disappeared but a more intense staining of the chromaffin cells was observed. This indicates that the heptapeptide has a dual localization in the adrenal medulla and the synthesis and/or secretion of the peptide is under neuronal control through the splanchnic nerve. In normal adrenal glands, substance P-like immunoreactivity was found in varicose fibers in the medulla either as bundles or single fibers. After denervation these fibers were no longer seen, but instead a granular immunofluorescence was observed in a few chromaffin cells. This indicated that substance P also has a dual localization in the adrenal medulla. Few varicose fibers in the adrenal medulla exhibited bombesin-like immunoreactivity in normal rats. No immunofluorescence was observed in denervated glands. The possible localization of substance P and bombesin in the splanchnic nerve fibers remains to be studied as well as the possible effect of bombesin of catecholamine secretion of the chromaffin cells.

In the rat lung, substance P and bombesin were both localized in varicose fibers around small bronchioli. Met⁵-enkephalin-Arg⁶-Phe⁷ was found in numerous cells in the lung around small bronchioli or as groups of 20-40 cells. The immunofluorescence in these cells was granular and the cells are most probably the endocrine cells of the lung. Nerve fibers in the lung did not exhibit immunoreactivity. The lung appears to be rich in several neuropeptides and the interrelations of these peptide containing systems are currently being investigated.

The peptides included in this study act as neurotransmitters or neuromodulators in the central and peripheral nervous systems and are involved in mediation of pain sensation from the periphery through the spinal cord to higher centers of the brain. They are also involved in the thermoregulation, control of autonomic nervous system and hormone secretion in the hypothalamus and other organs. The exact localization of the peptides is a necessary step for a better understanding of the neuronal pathways which regulate these physiological functions and disorders of the sensory system and endocrine organs. Future research will involve pharmacological studies on specifically identified peptidergic neuronal systems whose regulation is still largely unknown.





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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01559-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Met ⁵ -enkephalin-arg ⁶ -phe ⁷ in the brain | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | H.-Y.T. Yang E. M. Majane J. Tang P. Panula E. Costa | Pharmacologist Chemist Visiting Fellow Visiting Fellow Chief |
| | | SMRP SMRP SMRP SMRP SMRP |
| | | NIMH NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.7 | PROFESSIONAL: 0.7 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Distribution and characteristics of <u>met⁵-enkephalin-arg⁶-phe⁷</u> (YGGFMRF) in the rat <u>brain</u> was studied by radioimmunoassay. The YGGFMRF is unevenly distributed in the brain with the highest content in striatum and hypothalamus and the lowest in the hippocampus and cerebellum. The distribution of YGGFMRF is very similar to that of <u>met⁵-enkephalin</u> . However, the content of YGGFMRF is smaller than that of <u>met⁵-enkephalin</u> in every region studied. The YGGFMRF can be <u>released from striatal slices</u> by depolarizing concentration of KCl in a Ca ⁺⁺ dependent manner raising the possibility of a <u>neuoregulatory role</u> for YGGFMRF. The YGGFMRF immunoreactivity in striatum and hypothalamus is composed mainly of authentic YGGFMRF. However, <u>multiple forms</u> of immunoreactive material, which include high molecular weight forms, exist in some brain regions. Further characterization of high molecular weight YGGFMRF is in progress. The uneven distribution of YGGFMRF suggests that this endogenous opioid peptide may participate in neuronal transmission in brain function. The possible interaction between dopamine and YGGFMRF in striatum will be studied using specific drugs. | | |

Project Description:

In this study, the distribution and characteristics of met⁵-enkephalin-arg⁶-phe⁷ (YGGFMRF) was studied. As a tool for the YGGFMRF assay, a specific antiserum against YGGFMRF was raised. The antiserum appears to be directed to the c-terminal portion of the YGGFMRF as it showed totally no cross-reaction with met⁵-enkephalin, met⁵-enkephalin-arg⁶, met⁵-enkephalin-arg⁶-arg⁷, met⁵-enkephalin-Lys⁸ and leu⁵-enkephalin. However it cross reacted with phe-met-arg-phe by about 10% and with the molluscan cardioexcitatory peptide, phe-met-arg-phe-NH₂, by an insignificant degree. The specificity of this antiserum made it possible for us also to study immunohistochemically the distribution of YGGFMRF-like immunoreactivity independently from met⁵-enkephalin.

The YGGFMRF-like immunoreactivity is unevenly distributed in the brain with the highest content in striatum and hypothalamus and the lowest in cerebellum. The regional distribution of this peptide is similar to that of met⁵-enkephalin, however, in every brain structure studied, the content of YGGFMRF is smaller than that of met⁵-enkephalin. The localization of YGGFMRF-like immunoreactivity, as revealed by immunohistochemical study, was also found to be similar to that of met⁵-enkephalin. This raises the possibility that these two peptides may be localized in the same neurons but not every cell, that contains met⁵-enkephalin, stained positively with the YGGFMRF antiserum.

Release of YGGFMRF was studied by perfusion of striatal slices. The YGGFMRF immunoreactive material was released in a Ca⁺⁺ dependent manner by a depolarizing concentration of KCl raising the possibility of a neuroregulatory role for YGGFMRF.

The characterization of the immunoreactivity by BioGel P-2 column chromatography followed by HPLC showed that the YGGFMRF immunoreactive material in striatum and hypothalamus is composed mainly of YGGFMRF. However, in other brain regions, multiple forms of YGGFMRF immunoreactive material, which includes significant amount of high molecular weight form, were detected. The further purification and characterization of the high molecular weight YGGFMRF like peptide is in progress.

In this study, a specific radioimmunoassay method was developed for the endogenous opioid peptide, YGGFMRF, and distribution of this peptide in brain was analyzed. The uneven distribution of YGGFMRF suggest that this opioid peptide may play an important role in the neuronal function of the brain. The possible interaction between dopamine and this less well studied opioid peptide, YGGFMRF, will be explored pharmacologically.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01560-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Met ⁵ -enkephalin-arg ⁶ -phe ⁷ in peripheral tissue | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | J. Tang J. Chou P. Panula H.-Y.T. Yang E. Costa | Visiting Fellow Guest Worker Visiting Fellow Pharmacologist Chief |
| | | SMRP SMRP SMRP SMRP SMRP |
| | | NIMH NIMH NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 0.7 | PROFESSIONAL: 0.7 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The distribution of <u>met⁵-enkephalin-arg⁶-phe⁷</u> (YGGFMRF) in peripheral tissues was studied by a sensitive radioimmunoassay coupled with gel filtration and HPLC. The highest content was found in various parts of intestine, lung and superior cervical ganglia. The distribution of YGGFMRF is not parallel to that of <u>met⁵-enkephalin</u> in these tissues. The YGGFMRF can be released from the slices of the rat lung in a Ca ⁺⁺ dependent manner by 47 mM KCl. A high affinity <u>opiate receptor</u> was demonstrated in the rat lung membrane preparations using [³ H]-etorphine as opiate ligand. Immunohistochemically, the YGGFMRF immunoreactivity in the <u>lung</u> was localized in the APUD-like cells closely associated with the wall of small and medium sized <u>bronchioli</u> . These results suggest that YGGFMRF may play an important role in the function of respiration in the lung. The possible physiological role of YGGFMRF in lung will be further explored. | | |

Project Description:

The opioid peptide, met⁵-enkephalin-arg⁶-phe⁷ (YGGFMRF), was originally isolated from bovine adrenal gland and its presence in brain, although in much smaller quantity than met⁵-enkephalin, was subsequently demonstrated. This opioid peptide has been shown to behave like opioid agonist in many bioassay systems; however its physiological function still remains unclear. In searching for an appropriate system to explore the biological role of this opioid peptide, the distribution of the YGGFMRF in various peripheral tissues was studied.

The YGGFMRF content in various tissues of rats and guinea pigs was determined by radioimmunoassay after its isolation from the tissue extract by combination of gel filtration and HPLC. The YGGFMRF was found to be widely distributed in various peripheral tissues. In the rat, high contents were detected in duodenum, ileum, lung and superior cervical ganglia. In the guinea pig, high levels of YGGFMRF were detected throughout the intestine and also in the lung. In all the tissue studied, except in myenteric plexus of rats, the content of YGGFMRF was found to be greater than that of met⁵-enkephalin. Especially in the lung where a high level of YGGFMRF but an undetectable level of met⁵-enkephalin was observed.

Willet and Sapru (1982) suggested that enkephalin-like peptide probably exists in the lung, and this opioid peptide may act on the "J" receptor thereby triggering modification of breathing through reflex mechanisms mediated by phrenic and laryngeal nerves. In view of this report, the presence of YGGFMRF in the lung, as documented in this study, strongly suggests the importance of YGGFMRF in the respiratory function of the lung. Immunohistochemically, the YGGFMRF immunoreactivity in the rat lung was shown to be located in APUD-like cells closely associated with the wall of small and medium size bronchioli. The YGGFMRF immunoreactive material can be released from the rat lung slices by 47 mM KCl and this effect was abolished by the omission of Ca⁺⁺ from the perfusion medium. Using [³H]-etorphine as an opiate ligand, a high affinity opiate receptor was demonstrated in the rat lung membrane preparation. This etorphine binding can be displaced by YGGFMRF with a greater affinity than met⁵-enkephalin. These results allow us to speculate that YGGFMRF may be released from APUD-like cells and acts on specific opiate receptors in the lung to participate in the respiratory function.

Other neuropeptides, bombesin and substance P were also measured in the rat lung and were found to be 0.94 ± 0.14 and 1.9 ± 0.06 pmol/mg protein respectively.

The stimuli that release YGGFMRF and interaction of YGGFMRF with other neuroregulators including other neuropeptides remain to be explored.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01561-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Cholecystokinin in brain | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: M. J. Iadarola Other: H.-Y.T. Yang E. Costa | Guest Worker Pharmacologist Chief | SMRP SMRP SMRP NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The possibility that the <u>neuropeptide cholecystokinin</u> (CCK) was <u>co-localized</u> with <u>dopamine</u> (DA) was examined in rats with unilateral <u>6-hydroxydopamine</u> (6OHDA) or mechanical hemitransection of the <u>mesolimbic DA system</u> . The effects of the lesions on CCK content and tyrosine hydroxylase (TH) activity were evaluated in the olfactory tubercle (OT) and nucleus accumbens (NA) (representative of the meso-limbic system) and the caudate-putamen (CP) (representative of the nigro-striatal system). After hemisection the maximal loss of CCK was 50-60% in OT and NA; no change was observed in CP despite decreases of 85-97% in TH activity. Similar effects upon CCK and TH were observed with 6OHDA lesion placed in the <u>ventral tegmental area</u> ; whereas 6OHDA placed laterally in the <u>substantia nigra</u> had little impact on CCK content of either OT, NA or CP. These results suggest that: 1) a substantial proportion of the CCK content of OT and NA may be derived from DA neurons, 2) DA-CCK co-localization appears to be mainly a feature of the mesolimbic system rather than the nigrostriatal system and 3) the remaining CCK in OT and NA and the majority of CCK in CP derived from another, most likely cortical source. | | |

Project Description:

Immunocytochemical studies have suggested the coexistence of dopamine (DA) and cholecystokinin (CCK) in neurons of the substantia nigra ventral tegmental complex (SN-VTA), projecting to olfactory tubercle (OT) and nucleus accumbens (NA). Our initial effort was directed at verifying this association using biochemical and surgical techniques. We performed a series of mechanical hemitranssections and nigral or A-10 infusions of 6-hydroxydopamine combined with radioimmunoassay for CCK and assessment of tyrosine hydroxylase activity. Briefly, our results suggest that 1) about 50% of the CCK content of OT and NA is not associated with mesolimbic DA terminals; 2) there is small but significant contribution (to NA and OT) of cells lateral to VTA that contain DA but no CCK; 3) nearly 70% of the DA innervation of the OT and NA may contain CCK and 4) the striatal content of CCK appears to be largely independent of the DA projection to the nucleus.

Thus, the neurochemical topography of the DA-CCK colocalization appears to correspond best to the topography of the mesolimbic A-10 DA projection. Our short term objective, therefore, is to further define this system by examining other tissues known to receive a prominent A-10 innervation such as ventral anterior frontal cortex, anterior olfactory nuclei and septum in our sample.

Further studies will be directed at a more functional neurochemical characterization of this system and include 1) localization of the CCK receptor to pre- or postsynaptic elements in OT and NA followed by 2) an examination of the influence of CCK on DA-stimulated adenylate cyclase activity, DA receptor binding or DA uptake. Through these studies we may be able to ascertain, in central nervous tissue, the possible function(s) of a co-transmitter system and what role CCK may have in schizophrenic disorders.

Other projects, planned in collaboration with the clinical division, involve the measurement of CCK in post-mortem schizophrenic human brain tissue and in CSF samples from schizophrenics.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01562-01 SMRP | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) The cholinergic neuronal system | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">P. E. Potter</td> <td style="width: 30%;">Visiting Fellow</td> <td style="width: 15%;">SMRP</td> <td style="width: 10%;">NIMH</td> </tr> <tr> <td>Other:</td> <td>J. L. Meek</td> <td>Pharmacologist</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td></td> <td>N. H. Neff</td> <td>Chief</td> <td>SMRP-B</td> <td>NIMH</td> </tr> </table> | | | PI: | P. E. Potter | Visiting Fellow | SMRP | NIMH | Other: | J. L. Meek | Pharmacologist | SMRP | NIMH | | N. H. Neff | Chief | SMRP-B | NIMH |
| PI: | P. E. Potter | Visiting Fellow | SMRP | NIMH | | | | | | | | | | | | | |
| Other: | J. L. Meek | Pharmacologist | SMRP | NIMH | | | | | | | | | | | | | |
| | N. H. Neff | Chief | SMRP-B | NIMH | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | | | | | | | | | | | | | |
| SECTION Biochemical Pharmacology | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.3 | PROFESSIONAL: 1.3 | OTHER: 0 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input type="checkbox"/> (a) HUMAN SUBJECTS</td> <td style="width: 33%;"><input type="checkbox"/> (b) HUMAN TISSUES</td> <td style="width: 33%;"><input checked="" type="checkbox"/> (c) NEITHER</td> </tr> </table> | | | <input type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input checked="" type="checkbox"/> (c) NEITHER | | | | | | | | | | | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS | <input type="checkbox"/> (b) HUMAN TISSUES | <input checked="" type="checkbox"/> (c) NEITHER | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to identify and study the acetylcholine-containing neurons. Our present objective is to develop a <u>simple method</u> to <u>assay acetylcholine</u> and <u>choline in neuronal tissue</u> . | | | | | | | | | | | | | | | | | |

Project Description:

Acetylcholine was the first neurotransmitter to be described. Unfortunately it has not been investigated to the same extent as other transmitter substances because methods for its assay are either insensitive, complicated, require expensive reagents or require specialized equipment. Our goal was to develop an assay of acetylcholine that had none of the aforementioned disadvantages.

We have developed a rapid, simple method for acetylcholine and choline. The method is based on the separation of acetylcholine and choline by reverse phase HPLC and mixing the effluent with acetylcholinesterase and choline oxidase. Choline oxidase converts choline to betaine and hydrogen peroxide. Production of hydrogen peroxide is continuously monitored with an electrochemical detector. The assay takes about 10 min and the sensitivity for the detection of choline and acetylcholine is 1 and 2 pmole, respectively. The content found for sample of brain using this new procedure are similar to the content found by gas chromatography-mass spectrometry. HPLC with electrochemical detection offers advantages over conventional methods for determining acetylcholine. Both acetylcholine and choline can be measured in the same sample, yet the equipment used is common in many laboratories. Because no prior derivatization or separation of acetylcholine is required, sample preparation is rapid. The method is simple and reproducible and offers excellent sensitivity and specificity. Our procedure for measuring acetylcholine should allow more laboratories to study cholinergic neurons and perhaps gain a better understanding of their physiological role. Our future studies will be directed towards utilizing our procedure to understand cholinergic function and the action of drugs on the system.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01563-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Adenosine: A putative neurotransmitter | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | W. Wojcik N. H. Neff | PRAT Fellow Chief |
| | | SMRP SMRP-B |
| | | NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Biochemical Pharmacology | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.1 | PROFESSIONAL: 1.1 | OTHER: |
| CHLCK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to identify and characterize neuronal systems that utilize <u>adenosine</u> as a <u>transmitter</u> or <u>neuromodulator</u> . Our present objective is to provide evidence that adenosine serves a role in neuronal function within the <u>striatum</u> and <u>cerebellum</u> . | | |

Project Description:

Adenosine has been proposed as a neurotransmitter or modulator because it has biochemical, electrophysiological and behavioral actions in animals. Our objectives were as follows: I) to develop a sensitive assay for adenosine in neuronal tissue; II) to provide evidence that adenosine metabolism changes with neuronal activity or following selective brain lesions; and III) to characterize adenosine receptor systems.

- I. Adenosine reacts with chloroacetaldehyde to form a fluorescent product (see Z01 MH 01536-03 SMRP). By using an appropriate HPLC system the fluorescent adenosine derivative was separated and quantitated in samples of brain. As little as 100 femtomoles of adenosine can be assayed in a sample. Adenosine was found to be uniformly distributed in the brain of rats killed by focused microwave radiation to the head.
- II. When animals were killed by decapitation there was an uneven distribution of adenosine in brain. The highest content was found in the striatum and interestingly this is the only structure where an adenosine-stimulated adenylate cyclase system could be detected. Three brain lesions were performed to determine the site of adenosine formation and the site of adenosine-stimulated cyclase in striatum: injection of 6-hydroxydopamine into the medial forebrain bundle to destroy the dopaminergic pathway; injection of kainic acid directly into the striatum to lesion neuronal cell bodies; and surgical decortication to remove the cortical-striatal projections. Only kainic acid lesions eliminated the rise of adenosine after decapitation and the ability of adenosine to activate adenylate cyclase. Apparently the source of adenosine and the adenosine cyclase receptor system are associated with neurons intrinsic to the striatum.
- III. Adenosine has been found to interact with two different membrane bound receptors, a high affinity receptor associated with activation of adenylate cyclase, termed A_1 , and a low affinity receptor associated with activation of adenylate cyclase, termed A_2 . The A_2 receptor appears to be located primarily in striatum on neurons that are destroyed by treatment with kainic acid. In contrast to A_2 receptors, A_1 receptors are found throughout the brain. By testing for the presence of A_1 receptors in neurologically mutant strains of mice with specific lesions in cerebellum, we conclude that A_1 receptors are associated with Purkinje cell dendrites and/or granule cells in cerebellum.

Some investigators have suggested that anxiolytic drugs act at adenosine receptor sites. At present, however, the role of adenosine in brain function is unclear. Our studies are providing basic information needed to evaluate adenosine's role in brain physiology.

Future studies will be directed towards evaluating whether there is, indeed, a specific adenosine-containing neuronal system.

Publication:

Wojcik, W.J., and Neff, N.H.: Adenosine measurement by a rapid HPLC-fluorometric method: Induced changes of adenosine content in regions of rat brain. J. Neurochem., in press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER 201 MH 01564-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Control of GABA turnover in rat striatum | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | O. Giorgi J. L. Meek | Visiting Fellow Pharmacologist |
| | | SMRP SMRP |
| | | NIMH NIMH |
| | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Group on High Pressure Liquid Chromatography | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.1 | PROFESSIONAL: 1.1 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The control of motor behavior by the <u>striatum</u> involves the action of a variety of neurotransmitters, including <u>glutamate</u> and dopamine (afferents to the striatum), acetylcholine and GABA (interneurons) and <u>GABA</u> , substance P and possibly adenosine (efferents). To better understand control of GABAergic function we examined the effects on <u>GABA turnover</u> of injection into rat striatum of several agonists and antagonists of these transmitters. As an index of turnover, we injected <u>gabaculine</u> (an inhibitor of GABA synthesis) directly into the striatum, and measured the rate of GABA accumulation. Injection of excitatory amino acids (glutamate and kainic acid) increased GABA turnover. Injection of a glutamate antagonist (glutamate di-ethyl ester) produced the opposite effect, and blocked the action of glutamate. Local injection of a dopamine agonist also increased GABA accumulation. Cholinergic agonists (carbachol and oxotremorine) had no effect on GABA accumulation. | | |

Project Description:

GABA (gamma-aminobutyric acid) plays important roles in the control of motor function by the striatum. This compound is a transmitter for both a major efferent pathway to the striatum, and interneurons within the striatum that process inputs to that structure. One approach to studying the interactions of these neuronal systems is to examine changes in turnover of GABA produced by local injection of neurotransmitter agonists and antagonists. Major inputs to the striatum include an excitatory pathway (probably glutamatergic) from the cortex, and a dopaminergic pathway from the striatum. Transmitters involved with interneurons include GABA, acetyl choline and probably adenosine. The local injection approach has the advantage over the conventional systemic or intraventricular routes that the action of the drugs is restricted to the structure itself, and thus the likelihood of long distance effects is minimized. As an index of GABA turnover, we have chosen the rate of accumulation of GABA after local injection of gabaculine, a potent specific irreversible inhibitor of glutamate decarboxylase. Gabaculine, or gabaculine plus a test compound were injected stereotactically into the striatum. At intervals, rats were decapitated and GABA content measured in the part of the striatum injected by HPLC (high pressure liquid chromatography).

MAJOR FINDINGS

- 1) Destruction of the intrinsic striatal neurons with kainic acid caused parallel decreases in GABA content (to 45 % of control) and the rate of GABA accumulation after gabaculine (40% of control). The majority of GABA which accumulates after gabaculine therefore arises from neurons sensitive to kainic acid.
- 2) Local injection of excitatory amino acids (glutamate or kainic acid) caused a 40-60% increase in the rate of accumulation of GABA.
- 3) Injection of large doses of glutamate diethylester (a glutamate antagonist) into the striatum decreased GABA accumulation, and blocked the effects of glutamate when both were injected together.
- 4) Local injection of apomorphine (a dopamine agonist) increased the rate of GABA accumulation, indicating an excitatory effect. This result is consistent with previous work from this laboratory (Revuelta, Cheney and Costa) that acute administration of haloperidol (a dopamine antagonist) intraventricularly decreases GABA turnover in the striatum.
- 5) Local injection of oxotremorine and carbachol (cholinergic agonists) had no effect on GABA accumulation in the striatum. These results are consistent with previous results from this lab. that intraventricular injection of a cholinergic agonist (methacholine) or antagonist (scopolamine) do not affect GABA turnover in striatum.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01565-01 SMRP |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | |
| TITLE OF PROJECT (80 characters or less) Regulation of GABA _A and GABA _B receptor function | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | M. D. Majewska D. M. Chuang E. Costa | Visiting Fellow Chemist Chief |
| | | SMRP SMRP SMRP |
| | | NIMH NIMH NIMH |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Preclinical Pharmacology | | |
| SECTION Molecular Neurobiology | | |
| INSTITUTE AND LOCATION NIMH, ADAMIA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | |
| TOTAL MANYEARS: 1.3 | PROFESSIONAL: 1.3 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER | | |
| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Crude synaptic membrane of rat brain contains <u>GABA_A</u> and <u>GABA_B</u> receptors. We have examined whether <u>GABA_A</u> or <u>GABA_B</u> receptor sites or both are coupled to the recognition sites for <u>benzodiazepines</u> . The enhancement of Bmax of ³ H-GABA binding at 37° by <u>diazepam (DIZ)</u> was increased by <u>EGTA</u> in the binding assay. When the binding to <u>GABA_A</u> and <u>GABA_B</u> was differentiated by the presence of <u>baclofen</u> and <u>THIP</u> respectively, the binding to <u>GABA_A</u> was enhanced by DIZ but the binding to <u>GABA_B</u> was unaffected. Conversely the enhancement of <u>³H-flunitrazepam</u> binding by <u>GABA_B</u> occurs only when <u>GABA_A</u> receptor sites are free. Thus <u>GABA_A</u> but not <u>GABA_B</u> recognition sites are linked to the DIA recognition sites. In addition, we have found that in the crude synaptic membranes, various <u>membrane-rigidifying compounds</u> enhance the high affinity binding of ³ H-GABA, whereas <u>membrane-fluidizing factors</u> decrease the binding. Moreover addition of free fatty acids or depolarization with K ⁺ reduced the specific binding of ³ H-GABA to intact NB _{2a} cells. These results suggest that membrane-fluidity plays an important role in regulating GABA receptor function. | | |

Project Description:

Recently it was reported that crude synaptic membranes of rat brain contain a Ca^{2+} -dependent high affinity binding site for GABA. This subtype of GABA binding sites is labeled with ^3H -baclofen and has been termed GABA_B recognition site. THIP or isoguvacine fails to bind to GABA_B recognition site and selectively work on the GABA binding site which was termed GABA_A recognition site. The present study was undertaken to examine whether GABA_A or GABA_B sites, or both are coupled to the recognition sites for benzodiazepines. Binding of ^3H -GABA (20 nM) to GABA recognition sites located in frozen and thawed crude synaptic membrane of rat brain was conducted at 37°C in 50 mM Tris-HCl pH 7.1 in the presence of aminooxyacetic acid to block the activity of transaminases. The addition of 0.25 mM EGTA to the binding assay diminished the specific binding by about 50%; this effect could be fully reversed by the addition of Ca^{2+} (0.25-0.50 mM) to the EGTA containing mixtures. In normal conditions, diazepam (5 μM) enhanced ^3H -GABA binding by about 20-30%; this enhancement was increased to 60-80% when EGTA was present during binding assay. This EGTA-induced enhancement was also nullified by the addition of Ca^{2+} ; at 10 mM Ca^{2+} , diazepam failed to enhance ^3H -GABA binding. Our preliminary results show that in the presence of EGTA, only a low affinity binding component was detected and the addition of diazepam caused the appearance of a high affinity binding component. These EGTA effects were not found in a membrane preparation pre-treated with AgNO_3 . When binding to GABA_A and GABA_B was differentiated by binding ^3H -GABA in the presence of 40 μM of baclofen and THIP respectively, the binding to GABA_A receptor was inhibited by high concentration of Ca^{2+} (>1.0 mM), but the binding to GABA_B receptor was greatly stimulated by Ca^{2+} . These effects were very specific for Ca^{2+} among various divalent cations examined. Moreover it can be verified that ^3H -GABA binding to GABA_A receptor was increased by diazepam but the binding to GABA_B receptor was totally unaffected. In a converse experiment, we measured the enhancement of ^3H -flunitrazepam binding by GABA. This enhancement by GABA was virtually abolished when GABA_A receptor was blocked by bicuculline but not when GABA_B receptor was blocked by baclofen. Thus our results suggest that GABA_A but not GABA_B recognition sites are linked to the benzodiazepine recognition sites. The enhancement of benzodiazepine binding by GABA or the enhancement of GABA binding by benzodiazepines are mediated by GABA_A recognition sites.

In addition, we have studied the regulation of GABA receptor binding by changing the fluidity of the plasma membrane. In crude synaptic membrane treated with various compounds known to rigidify membrane such as cholesterol and lysolecithin, the high affinity binding of ^3H -GABA to GABA receptors was found to be enhanced. In contrast, membrane-fluidizing agents such as unsaturated fatty acids significantly decreased the binding of ^3H -GABA to isolated membrane preparations. Moreover we have found that in neuroblastoma NB_{2A} cells, addition of free fatty acids or depolarization with high K^+ reduced substantially the specific binding of ^3H -GABA to intact cells. These results may have important physiological and pharmacological implications. For example, in brain synaptosomes or slices, a number of neurotransmitters (such as acetylcholine, NE and glutamate) and depolarizing factors (such as high K^+ and veratridine) are known to activate phospholipase A₂. It is very likely that the products of this enzyme, free fatty acids and lysolipids generated by physiological stimuli may modulate the function of GABA receptors through a

modification of the membrane fluidity, if these changes in fluidity occur in the same or neighboring lipid domains where GABA receptors are located. Studies are now in progress to obtain more direct evidence in support of such a view.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 MH 01566-01 SMRP | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1981 to September 30, 1982 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Molecular mechanisms in the antidepressant action of (-)deprenyl | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">G. Zsilla</td> <td style="width: 25%;">Guest Worker</td> <td style="width: 15%;">SMRP</td> <td style="width: 15%;">NIMH</td> </tr> <tr> <td rowspan="3">Other:</td> <td>M. L. Barbaccia</td> <td>Visiting Fellow</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td>O. Gandolfi</td> <td>Guest Worker</td> <td>SMRP</td> <td>NIMH</td> </tr> <tr> <td>E. Costa</td> <td>Chief</td> <td>SMRP</td> <td>NIMH</td> </tr> </table> | | | PI: | G. Zsilla | Guest Worker | SMRP | NIMH | Other: | M. L. Barbaccia | Visiting Fellow | SMRP | NIMH | O. Gandolfi | Guest Worker | SMRP | NIMH | E. Costa | Chief | SMRP | NIMH |
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| | E. Costa | Chief | SMRP | NIMH | | | | | | | | | | | | | | | | |
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| LAB/BRANCH Laboratory of Preclinical Pharmacology | | | | | | | | | | | | | | | | | | | | |
| SECTION Molecular Neurobiology | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032 | | | | | | | | | | | | | | | | | | | | |
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| <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) (-)Deprenyl is a selective inhibitor of the monoamino-oxidase (MAO) type B. MAO B uses as preferential substrates phenylethylamine (PEA) and dopamine, while MAO A catabolizes norepinephrine, dopamine and serotonin. Nevertheless (-)deprenyl given to depressed patients at the dose of 5 mg per day, which causes a selective MAO B inhibition, relieved many of their symptoms without eliciting the strong side effects induced by others MAO inhibitors. The possibility that the therapeutic effect of (-)deprenyl occurred through the formation of one of its metabolites, <u>amphetamine</u> , or through the inhibition of MAO B was examined. Our results exclude both possibilities and suggest that (-)deprenyl may act as <u>antidepressant</u> by modifying the function of <u>serotonergic axons</u> . | | | | | | | | | | | | | | | | | | | | |

Project Description:

On the basis of its reported usefulness in the therapy of depression we decided to investigate if (-)deprenyl interacted with the receptor function of various putative neurotransmitters in rat brain. In particular we were interested in looking at the effects elicited by deprenyl on noradrenergic and serotonergic receptors. Typical and atypical antidepressant drugs, when given for two weeks or longer, decrease the responsiveness to NE stimulation of the β -adrenergic receptor linked to a cAMP generating system, many of them decrease the number of binding sites for specific β -adrenergic antagonists and the number of 5HT₂ recognition sites in cortex and hippocampus of rat brain. The brain from various species, contains specific recognition sites for typical and atypical antidepressants such as imipramine and mianserin. A great proportion of ³H-imipramine and ³H-mianserin recognition sites appear to be anatomically and functionally related to the serotonergic system. Lesion studies have indicated that ³H-mianserin binding sites are mostly located on cell bodies on the postsynaptic site of serotonergic synapses while ³H-imipramine recognition sites are in great proportion located on 5HT axon terminals and they are functionally related to the 5HT uptake mechanism. Previously we have shown that the number of specific ³H-imipramine recognition sites can be decreased by daily imipramine or desmethylimipramine (DMI) injections repeated for two to three weeks. The latency time for this action is similar to that for the down regulation of the β -adrenergic receptors and for the appearance of the therapeutic efficacy of the antidepressants. For this project we decided to study the modifications of biochemical parameters induced by daily subcutaneous injections of (-) deprenyl (0.25 mg/kg per day for 21 days). This close schedule specifically inhibits MAO B in rats. This (-)deprenyl treatment decreased NE stimulation of the cAMP accumulation in slices from frontal cortex. The extent of desensitization was very closed to the one elicited by pargyline (which at 20 mg/kg i.p. for 21 days inhibits both MAO B and A). However, unlike pargyline, (-)deprenyl treatment failed to modify the number of β -adrenergic recognition sites as measured by ³H-dihydrolaprenolol (³H-DHA) binding to membrane prepared from rat cortex, nor changed the specific binding for ³H-spiroperidol to 5HT₂ receptors. When we examined the recognition sites labeled by ³H-imipramine and ³H-mianserin we found that repeated deprenyl injections induced a significant increase in the Bmax of ³H-imipramine binding sites in cortex and hippocampus while ³H-mianserin binding was unchanged. We asked whether the effect of (-)deprenyl on imipramine recognition sites could be a consequence of its capability to be metabolized into amphetamine. The answer was negative: an equimolar treatment with (+)amphetamine for 21 days failed to elicit any change in imipramine binding.

Our present results suggest that the pharmacological profile of (-)deprenyl is similar to that of antidepressants but different from that of MAO inhibition. It seems important to study the action on the imipramine binding because it expresses its capability to modify 5HT axon terminal function and perhaps the uptake of 5HT. Further studies are in progress to elucidate whether this action may be underlying the antidepressant action of (-)deprenyl.

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